Research Progress of the Southern California Particle Center: Health and Mechanism Studies

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Cardiovascular risks from fine particulate air pollution

- The study reported found an increased relative risk of 1.76 for death from coronary heart disease for every 10 ug/m3 in the mean concentration of PM2.5.
- The earlier ACS study predicted 1.12-1.18.
- If studies can identify intrinsic factors that lead to increased adverse cardiovascular disease then it should be possible to offer focused interventions to persons at greatest risk.
- This seminar will address how to identify intrinsic factors. These findings in post-menopuasal women are extremely serious.
- PM2.5 contains ultrafines, so the issue of which are the causative agent(s) is crucial

New England Journal of Medicine, 2007, 356, 447-458

History of the PM Center

- California Air Resources Board (ARB) support for development of particle concentrator and application to human clinical and animal toxicology studies (five years)
- Support from U.S. EPA for creation of a particle center
- Support from U.S. EPA for development of Supersite program (JF writing final document on health benefits from Supersite)
- Support from South Coast Air Quality Management District for research on the relation between air pollution and asthma (Asthma Consortium)
- Renewal of the PM Center for an additional five years (2005-2010)

Summary of PM Center accomplishments

- Atmospheric chemistry has a significant effect on PM composition
- Ultrafine particles play an important role
- A wider range of target tissues and health endpoints are associated with PM exposure than was known in 1997 including developmental effects, exacerbation of atherosclerosis and asthma; potential neurological consequences
- Results from diverse types of studies has strengthened the evidence that mobile sources are highly relevant to the public health risks posed by ambient PM
- Improved mechanistic understanding of PM toxicity has evolved

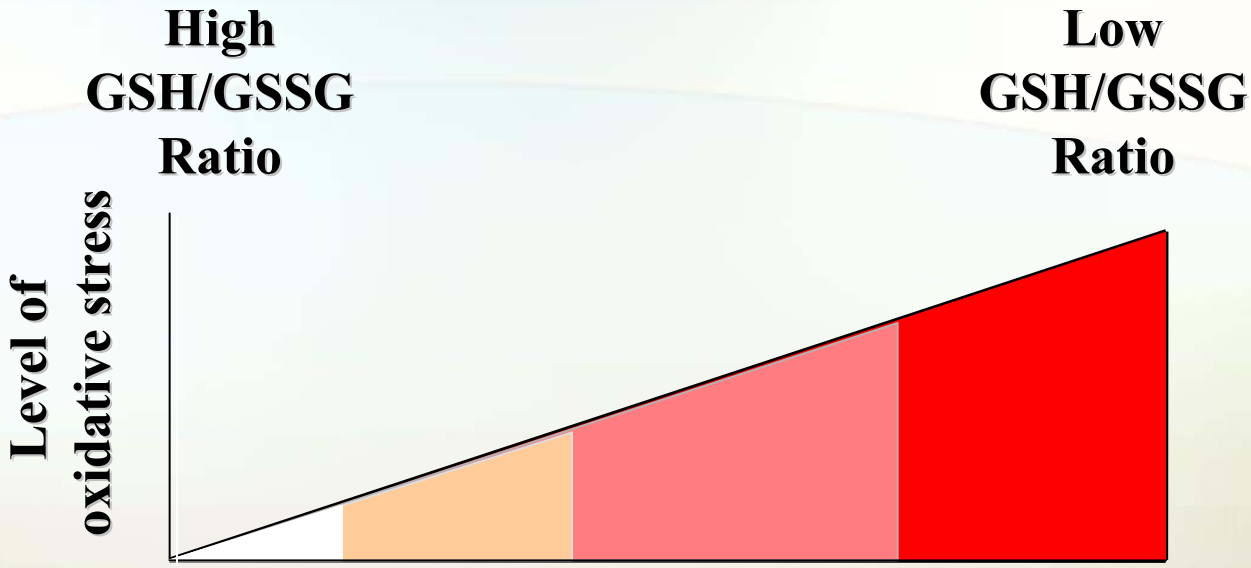
Mechanistic hypotheses for studying PM toxicity

- PM contains pro-oxidative chemicals
- Organic chemicals and metals located on the PM matrix are responsible for toxicity
- PM generates ROS and electrophilic chemistry→ oxidative stress including impact on cell signaling pathways
- The PM matrix is also capable of oxygen reduction
- Oxidative stress → Pro-inflammatory effects

Mechanistic hypotheses for studying PM toxicity

- Inflammation affects pathophysiology of asthma, cardiovascular disease and other endpoints
- Failure in antioxidant defense plays a role in susceptibility to PM-induced adverse health impact
- Genetic factors play an important role in toxicity
- Irreversible chemical reactions result chronic toxicity as a result of steady state exposure

Pathways of Oxidative Stress



Early view of the mechanistic pathways-very useful

Source: Xiao, et al. 2003

Approach to disease pathways based on sources of emissions

- Characterize physical/chemical properties of PM and vapor phase co-pollutants in relation to sources
- Use chemical assays to quantitatively assess PM and vapor characteristics in relation to initial stages of toxicity
- Link biological assays with progression of toxicity in cellular systems
- Conduct in vivo studies to confirm predicted outcomes based on mechanistic inference and chemical/biological assays
- Conduct human clinical and epidemiological studies to confirm pathways
- The objective is to develop a coherent roadmap to explain toxicity

Polycyclic aromatic hydrocarbons in the LA Basin

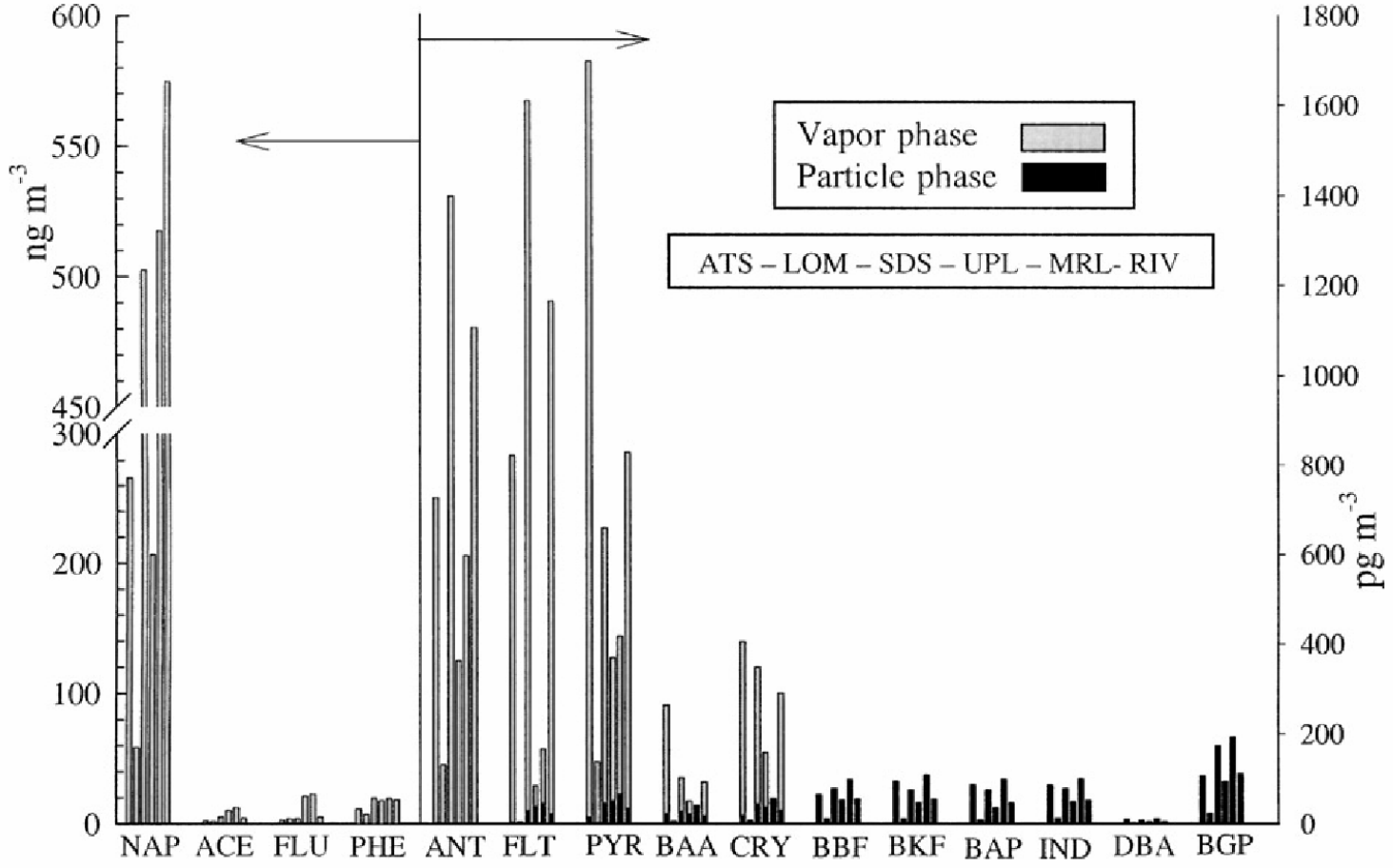


Figure 2. Annual averages for total PAH concentrations (vapor + particle phase) for individual species.

Eiguren-Fernandez et al., 2004

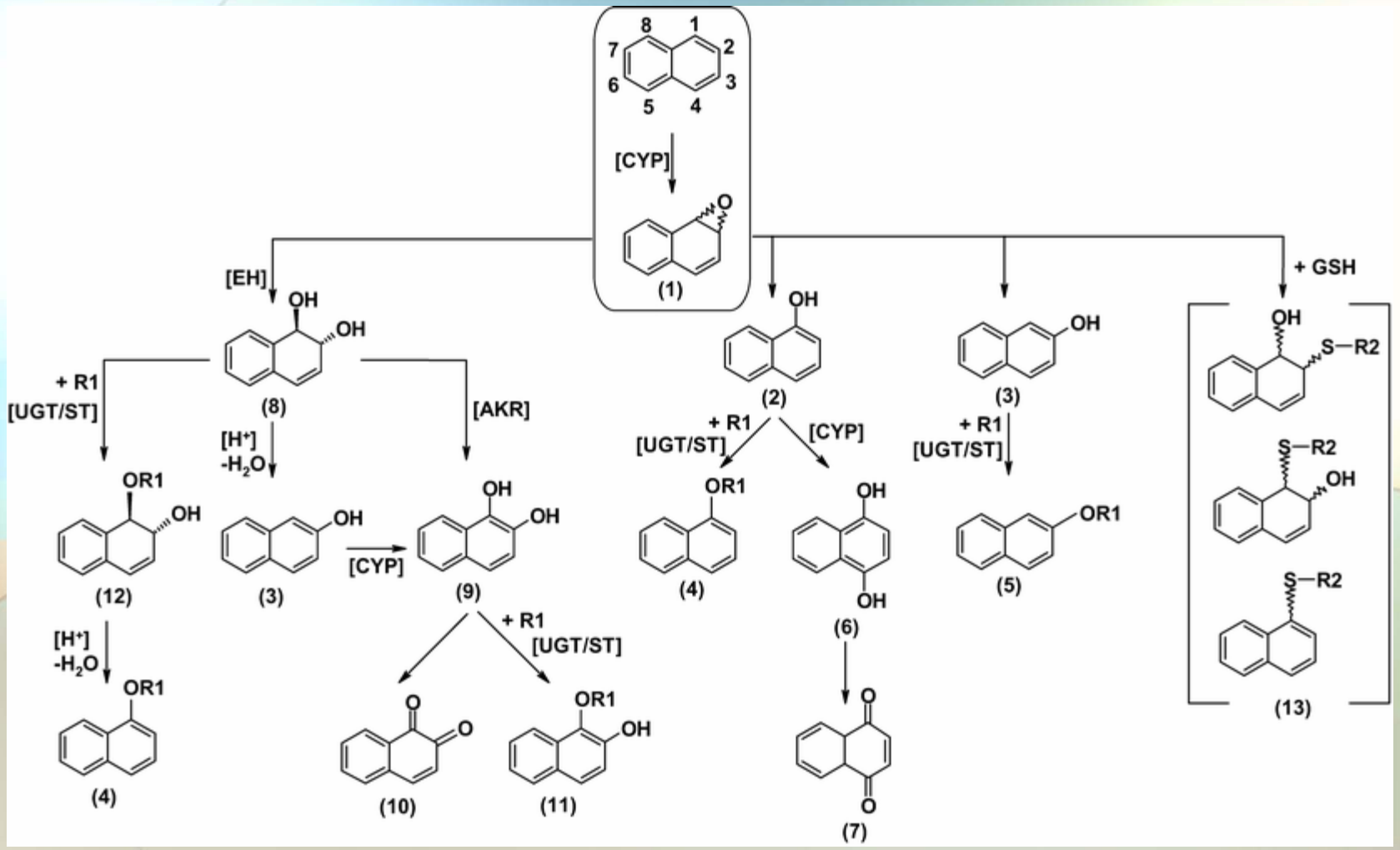
Annual PAHs - vapor (ng/m3) vs. PM(pg/m3) Riverside

Naph(V)	Phen(V)	BAP(PM)	BgP
575	18.5	47	112
Ratio napth/other PAHs	32	12,234	5133

Vapor phase PAHs account for approximately 95% of all PAHs

Naph levels as high as 5000-6500 ng/m3 were found in Mira Loma and Riverside

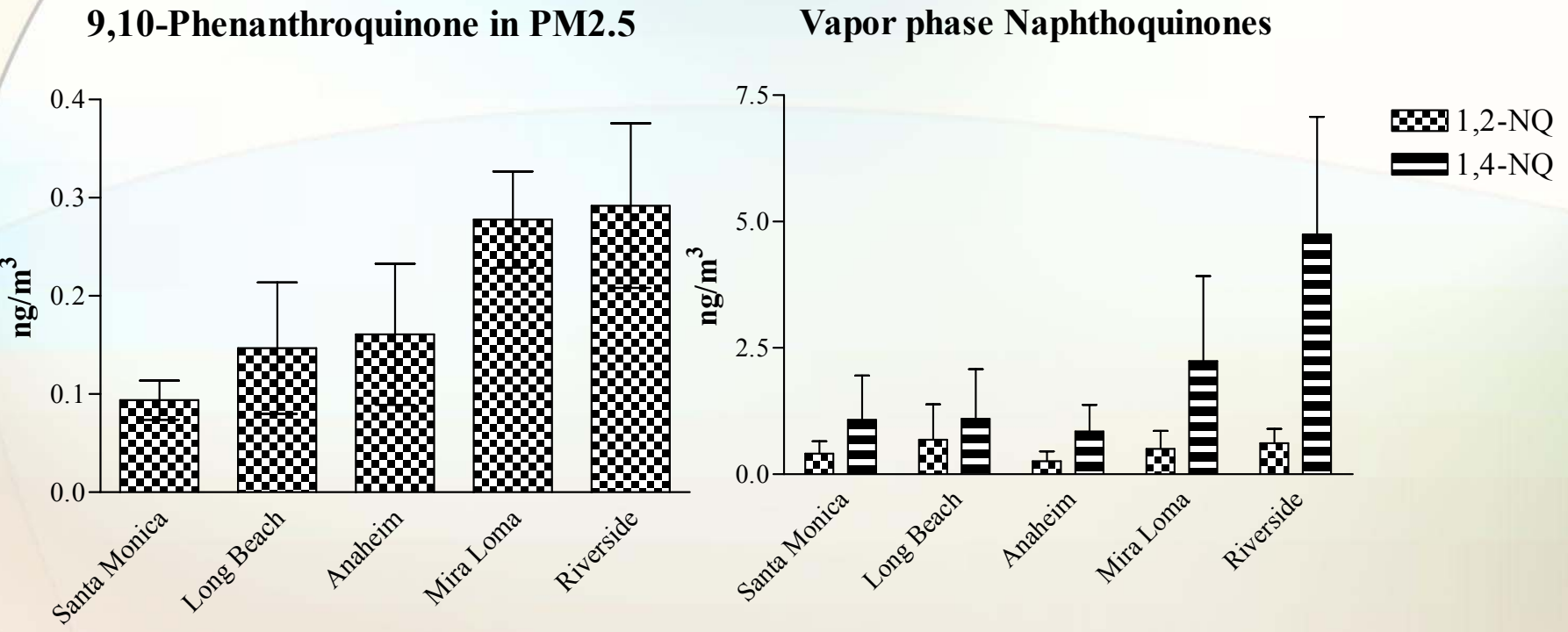
PAHs do not cause toxicity they are surrogates for other activies compounds



Gilliland (CHS) has shown fast metabolizers of naph are at greater risk of asthma which is consistent with formation of highly reactive quinones-demonstrates role of genetic susceptibility

Preuss, et al., (2003) Int Arch Occup Environ Health

Quinone content in ambient air

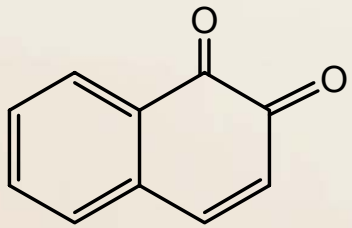


1. Note difference in scale of Y axes
2. Increase in 9,10-PQ, 1,4 NQ reflective of photochemistry
3. Prevailing wind is easterly.
4. PM 2.5 collected with Teflon filter, XAD resin (volatiles)
5. Collections followed air parcel based on wind velocity.

Miguel 2005

1,2-Naphthoquinone (1,2-NQ)

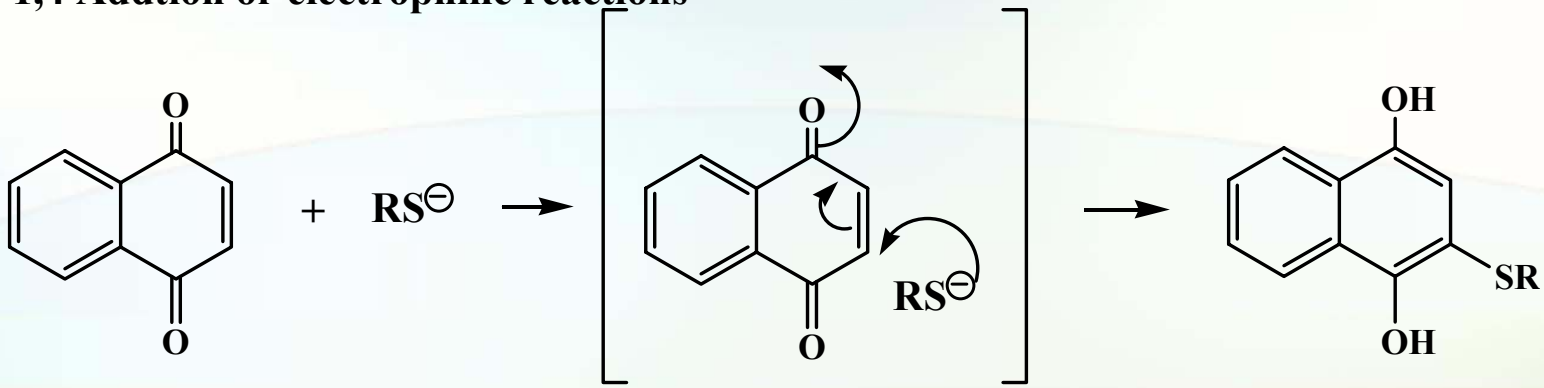
- Has both redox and electrophilic properties.
- Present in volatile and particle fractions of ambient air, on diesel exhaust particles.
- 1,2-napthoquinone produces contraction of smooth muscle, remodeling of lung cells, mucin production leading to exacerbation of asthma
- Potential for chronic effects that could be cumulative.



1,2-Naphthoquinone

Electrophilic properties of PM and vapors

1,4 Addtion or electrophilic reactions



- Formation of covalent bonds with cellular nucleophiles. Thiols are most available.
- Irreversible bond formation so once formed with cellular nucleophiles, recovery is based on resynthesis.
- Irreversibility can result in accumulation of effects, depending on the turnover of the affected species.
- The reaction does not require oxygen.

1,2-NQ: Contraction of smooth muscle

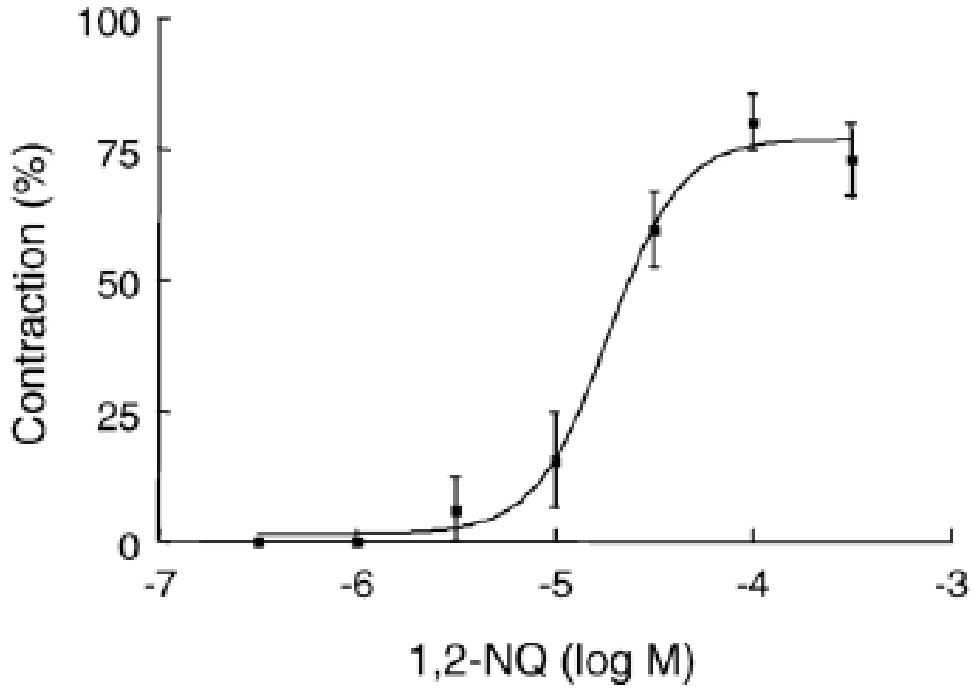
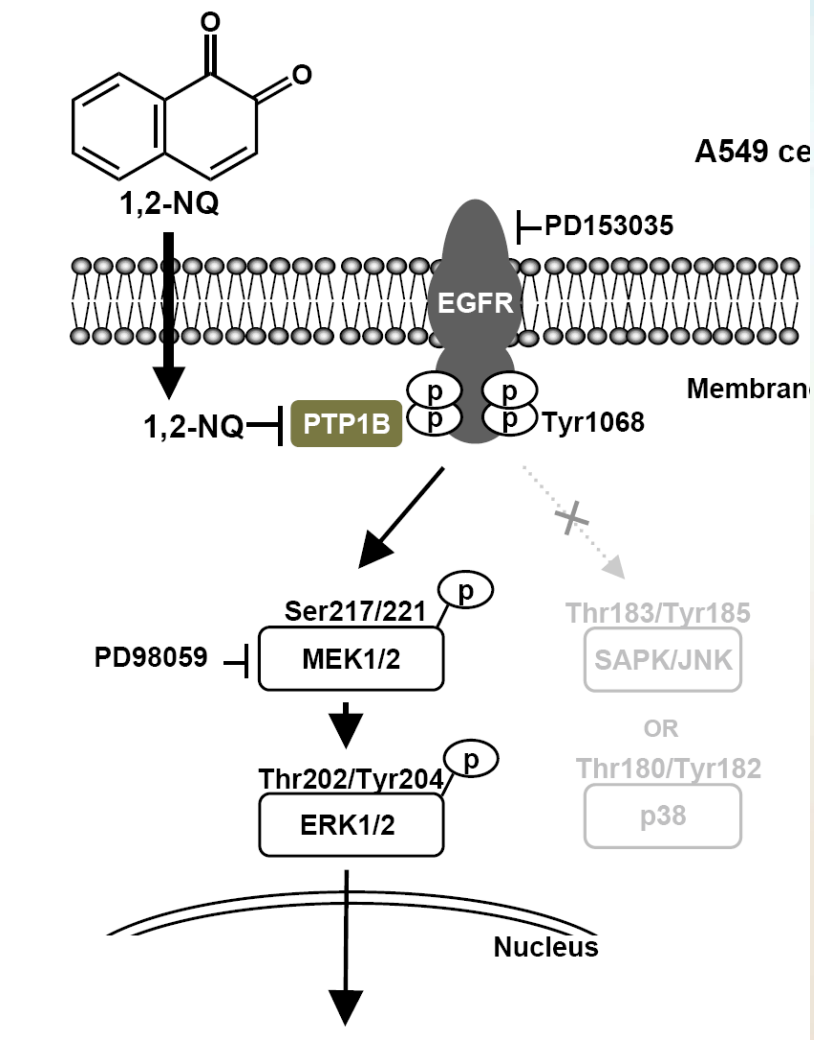


Fig. 1. Concentration-dependent contraction of guinea pig trachea by 1,2-NQ. Tracheal rings from guinea pigs were incubated with increasing concentration of 1,2-NQ. Results were calculated as the percent of the prior maximum response to KCl (40 mM) and given as mean ± SEM (n = 6).

Kikuno et al.

Toxicology and Applied Pharmacology 210 (2006) 47 – 54

1,2-Naphthoquinone and the EGFR system



Proposed interaction:

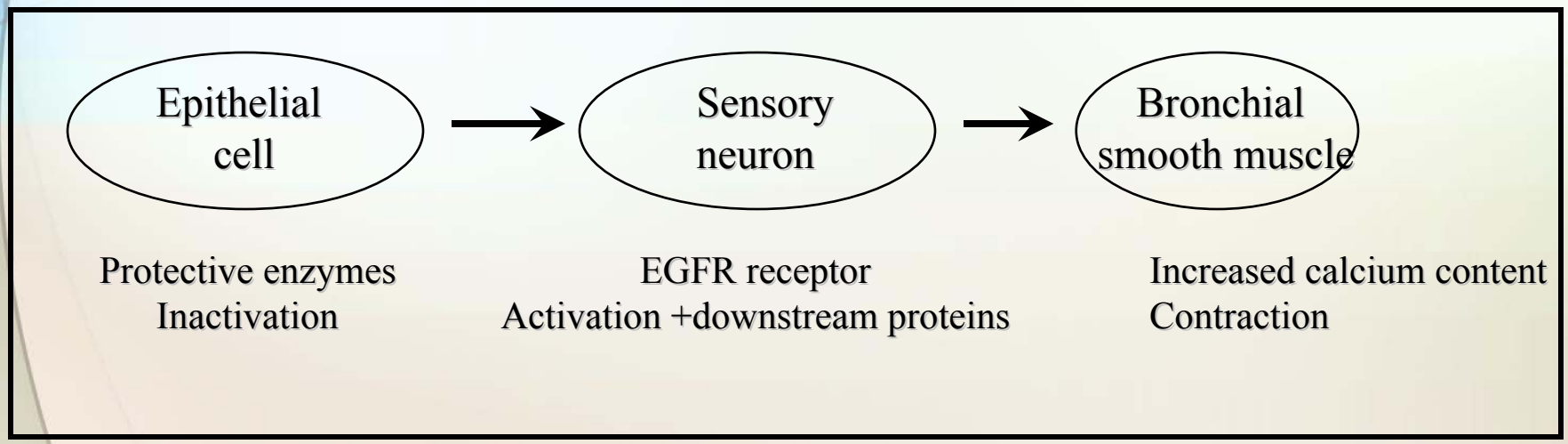
- Covalent attachment to PTP1B removes its inhibitory activity on EGFR.
- EGFR is phosphorylated and becomes activated, stimulating phosphorylation of down stream proteins.

Exacerbation of asthma by PM components from fossil fuel combustion

Napthoquinone (NQ) and other reactive compounds in PM

Naphthalene is most prevalent PAH in LAB; it is transformed to NQ in the air

Bronchia



•1,2-NQ actions are initiated by an irreversible effect and EGFR may be activated for a long time period.

•These actions could be cumulative during chronic exposure to low levels of this and other electrophilic quinones found in PM and in vapors.

Reduction in Airway diameter
exacerbation of asthma

Chemical assays

Goal: Quantitatively characterize the first steps in the chemistry of toxicity based on knowledge of chemical reactivity of vapor and PM constituents

- Electrophilic assay-irreversible chemistry between components and macromolecules (proteins, GADH, PTP1B, DNA)
- The ability of the sample to catalyze electron transfer from reducing source such as DTT or ascorbate to oxygen to generate ROS.
- DHBA-The ability of a sample to catalyze the generation of hydroxyl.

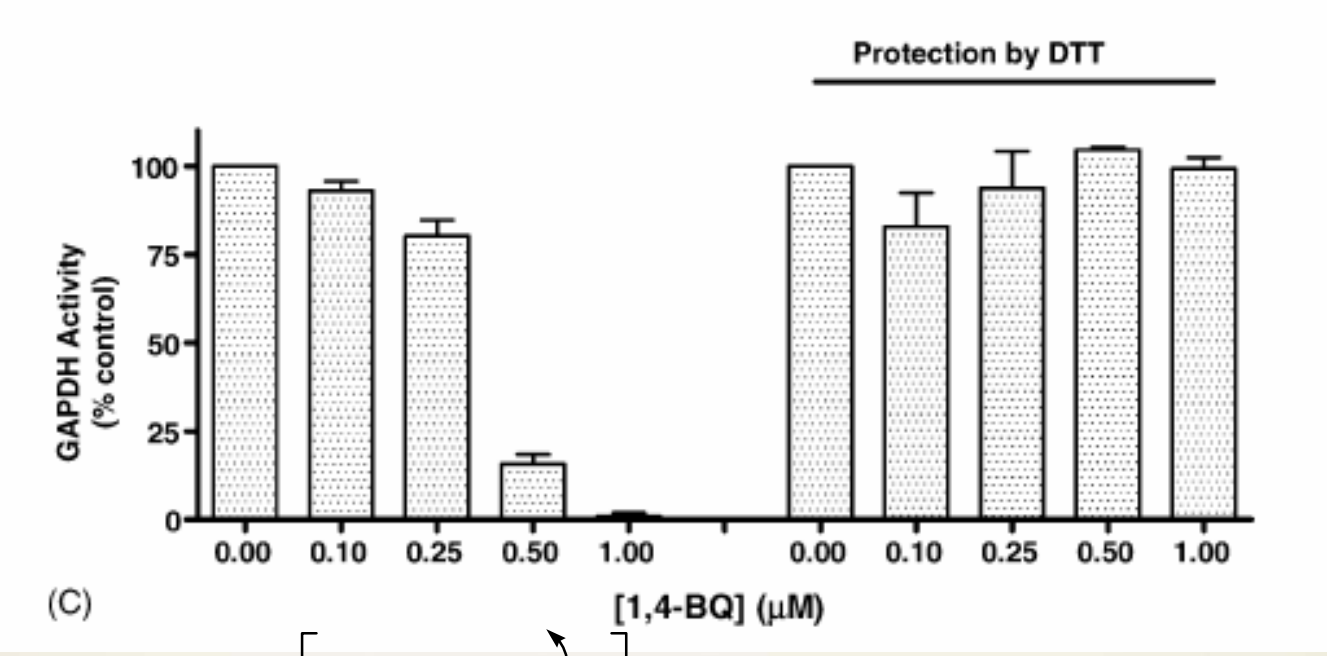
Electrophile assay

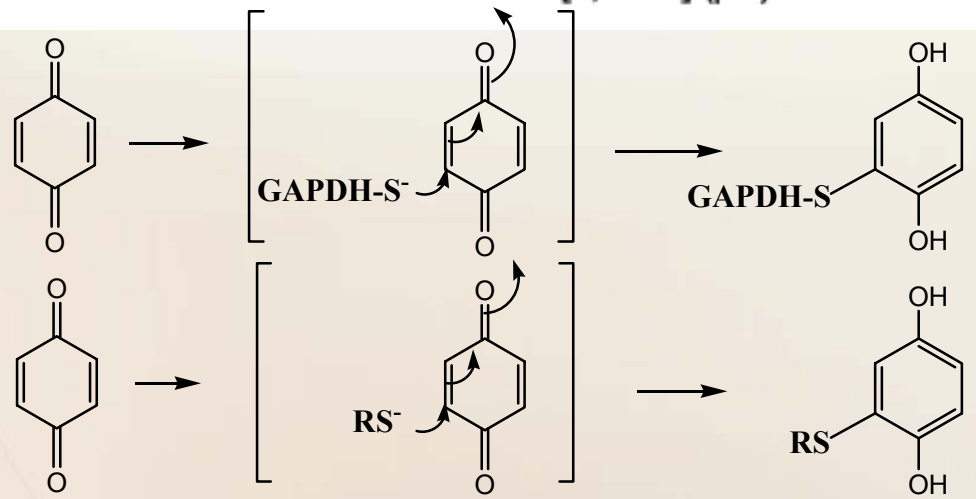
- PM contains electrophilic components such as the 1,2-napthoquinone
- Establish a routine assay that can analyze PM for electrophilic activity and link to sources.
- Application to ambient particle samples as a complement to redox assays.
- Then, we should be able to monitor chemical properties of PM for:
  - Organic based redox activity
  - Metal based redox activity
  - Electrophiles

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a nucleophile

- Protein of high concentration in most cells and is inactivated by electrophiles such as iodoacetic acid, acrylonitrile and a metabolite of acetaminophen through covalent bond formation.
- The covalent bond is formed between a thiolate function in the active site and the electrophile.
- The extent of covalent bond formation can be monitored by measuring enzyme activity.

BQ/electrophile and GAPDH



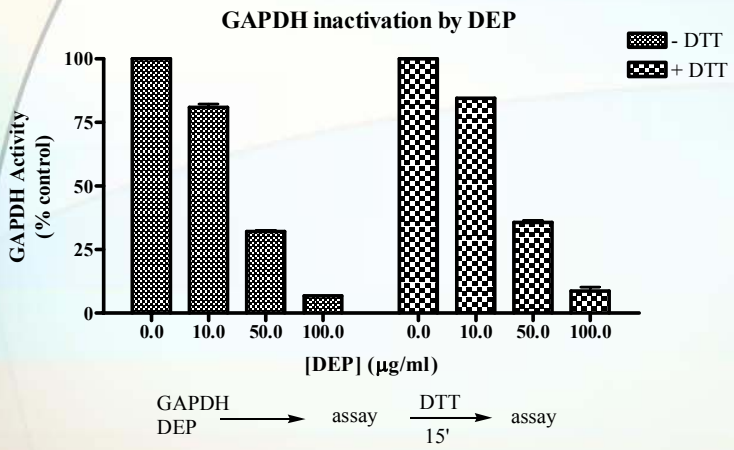


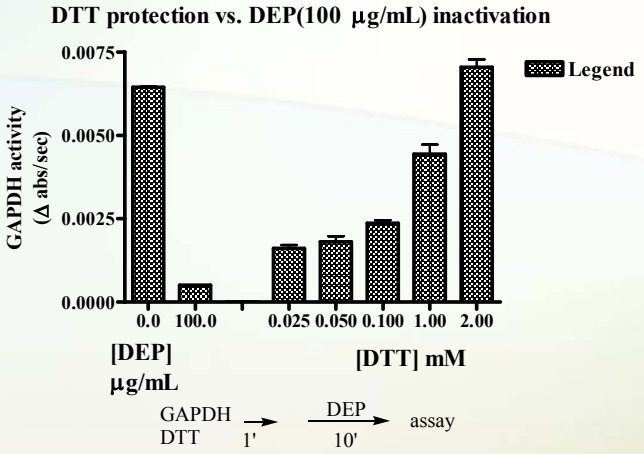
Inactivation by arylation

Protection by excess thiol

Rodriguez et al, in preparation

DEP extract and GAPDH

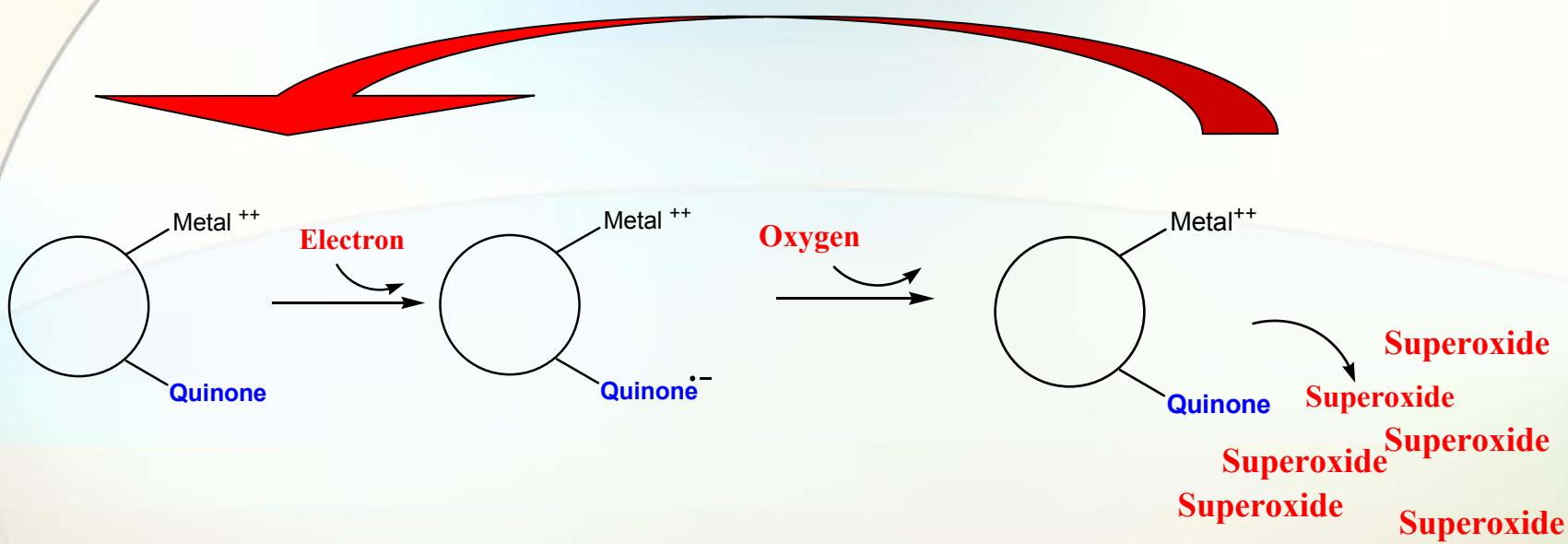


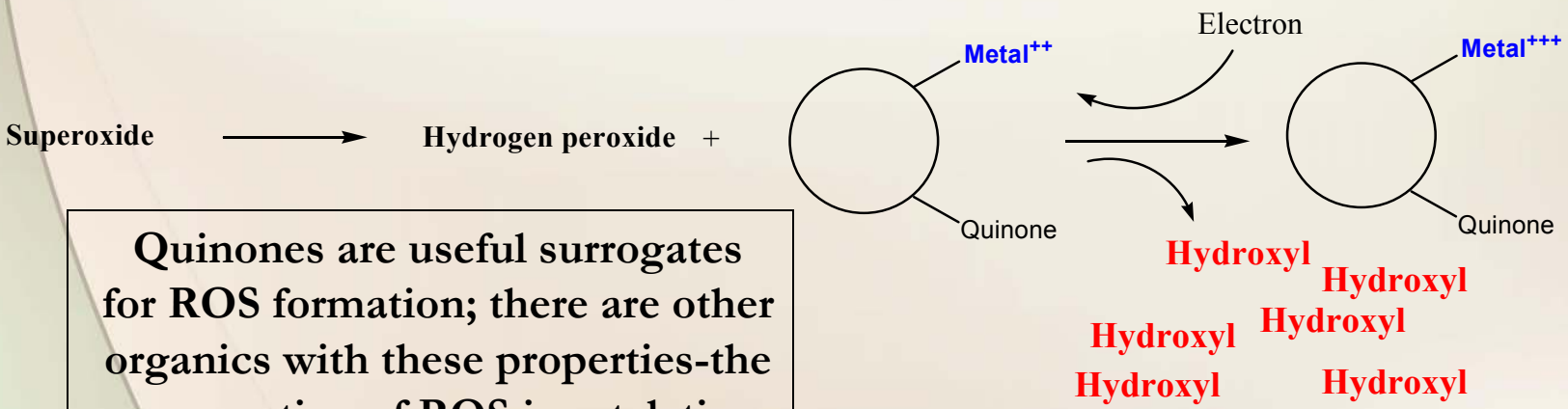


- Anerobic incubation of DEP with GAPDH results in time and concentration dependent inactivation.
- The inactivation can be protected by coincubation with a thiol (DTT) but can not be reversed by addition of DTT after inactivation
- Therefore, the inactivation is irreversible, consistent with covalent bond formation by an electrophilic species present in DEP.

Rodriguez, in preparation

Compounds Capable of Catalytic Redox Activity and Oxidative Stress Production





Quinones are useful surrogates for ROS formation; there are other organics with these properties-the generation of ROS is catalytic

Exposure to PM (BaP)

- We have developed assays to quantitate quinones in the air
- Metabolic processes are also present that convert protoxins to toxins

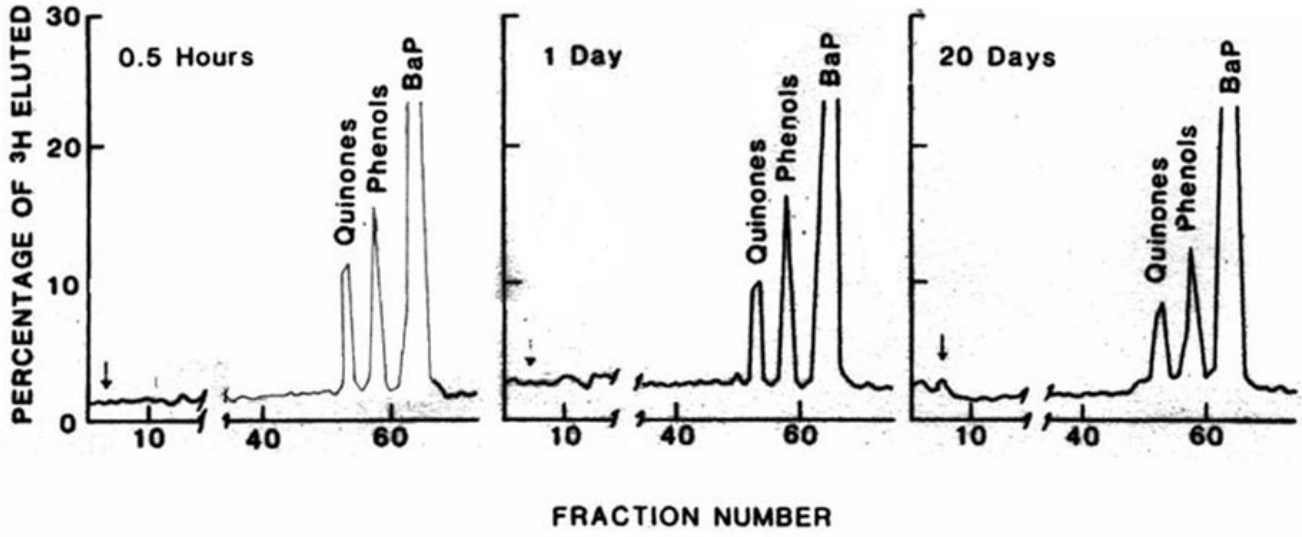
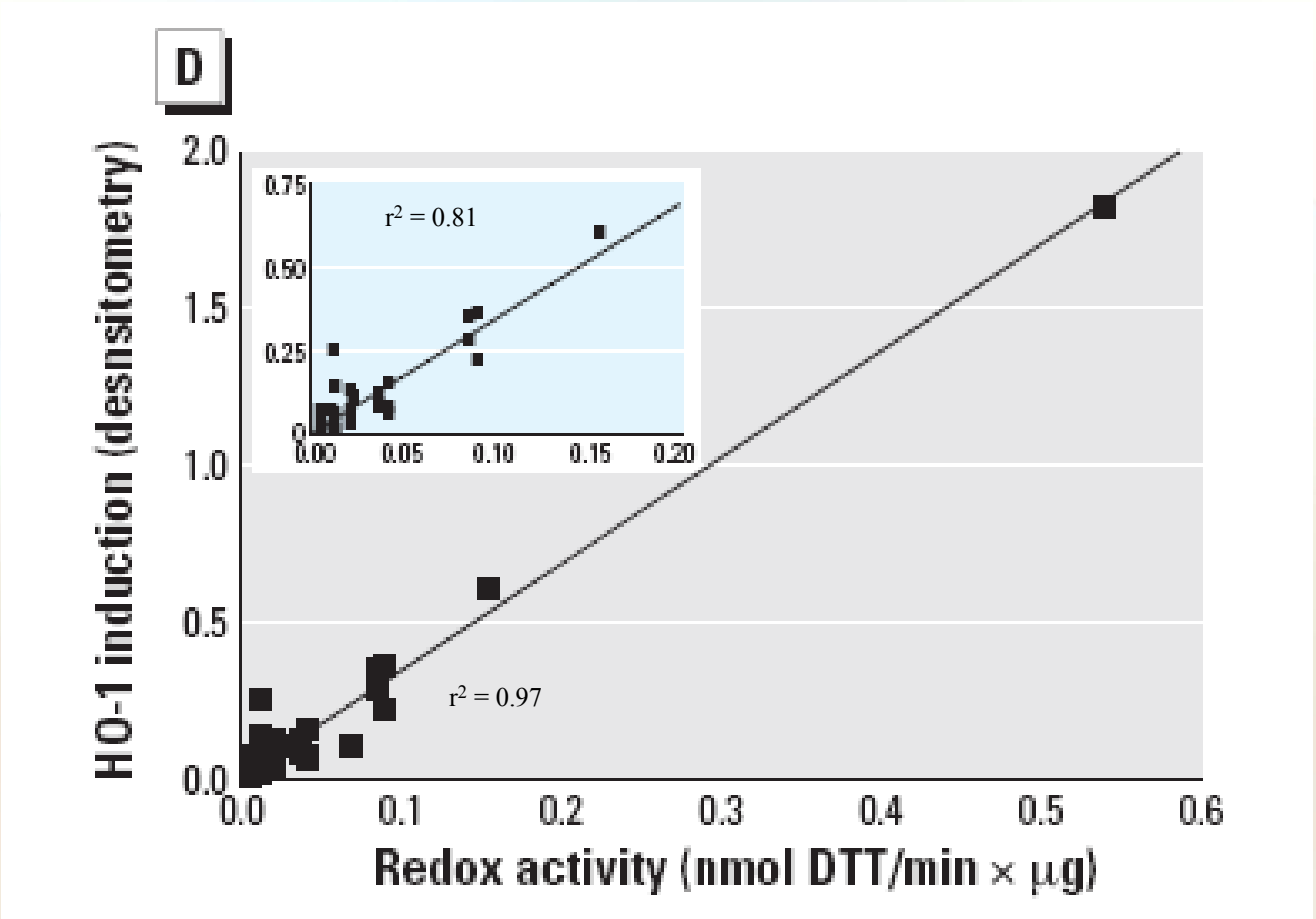


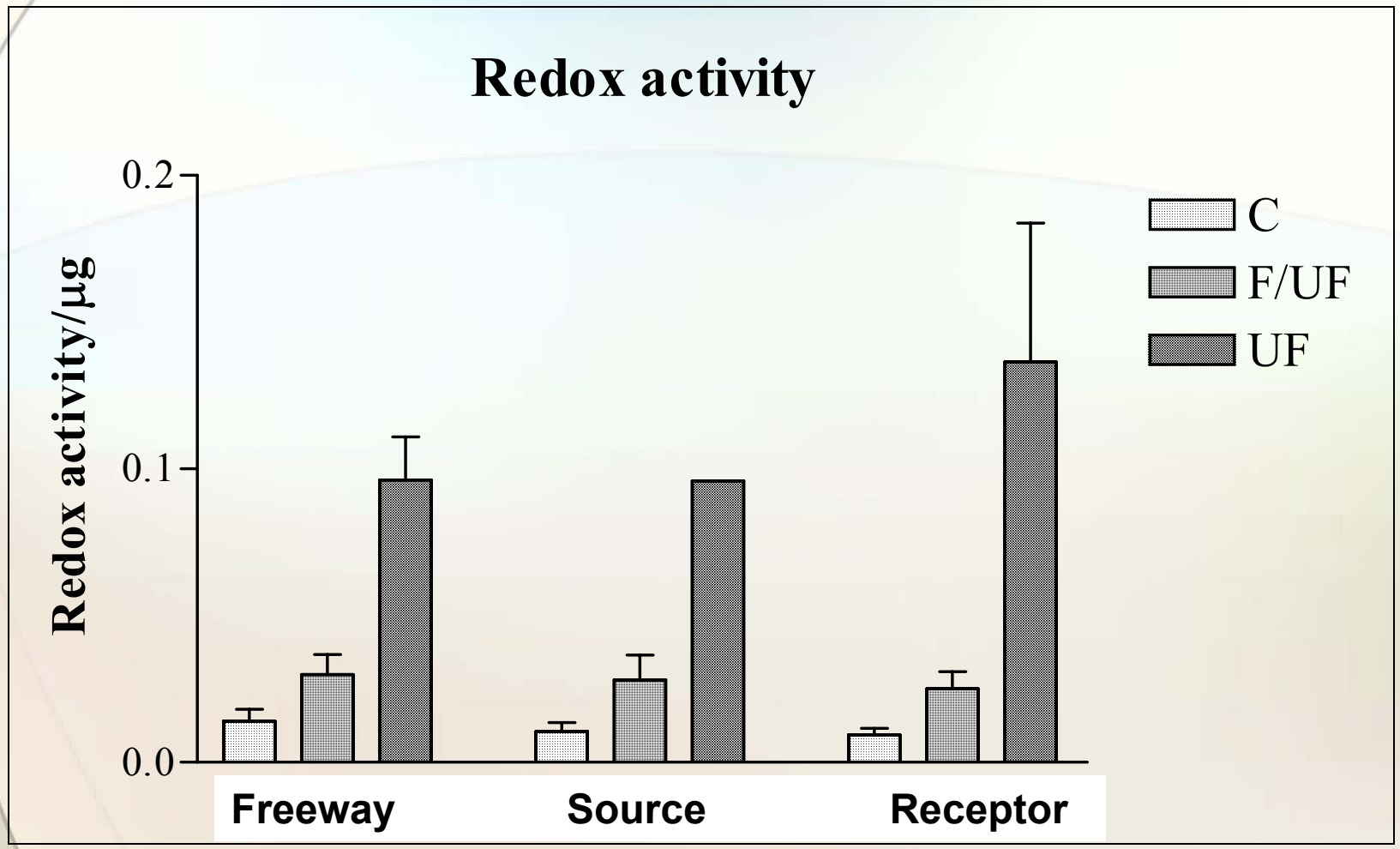
FIG. 6. High-performance liquid chromatographs of ^{3}H-BaP and ^{3}H-BaP metabolites organically extracted from lungs taken at various times after nose-only inhalation exposure of rats to diesel soot-associated ^{3}H-BaP. The arrow indicates the void volume. ^{3}H-BaP and its metabolites were identified by the comigration of ^{3}H radioactivity with authentic BaP standards detected by uv absorbance (254 nm).

Source: Sun et al., 1984

DTT based redox activity and HO-1 induction



Redox activity of ambient PM: effect of location and size fraction



Sample Availability

Location	Size		
	UF (<0.18um)	F+UF (<2.5 um)	Coarse (>2.5 um)
USC	1	–	–
Downey	1	2	–
Riverside	2	3	–
Caldecott B1	1	1	1
Caldecott B2	1	1	1
CA110	1	1	1

In total: 18 samples in 6 locations and three different size modes

DTT (nmols/min/μg) per location and size mode

Location	Size Modes					
	UF	**UF**	**F+UF**	**F+UF**	**F+UF**	**C**
USC	**0.061**					
Downey	**0.083**		**0.036**	**0.021**		
Riverside	**0.052**	**0.053**	**0.027**	**0.028**	**0.026**	
Caldecott B1	**0.111**		**0.068**			**0.019**
Caldecott B2	**0.172**		**0.075**			**0.032**
110FWY	**0.042**		**0.025**			**0.017**
Mean	0.082		0.038			0.023

Pearson DTT Correlations to Metals, Cr Correlations

Species	DTT
DTT	1.00
EC	0.26
OC	0.12
OC (excluding two unrealistic values)	***0.87***
Nitrates	-0.45
Sulfates	-0.08
Metals	-0.19
PAH 202-228 (FLU, PYR, BaA, CHR)	0.57
PAH 252 (BkF, BbF, BaP)	***0.92***
PAH 276-278 (BghiP, IcdF, dBahA)	***0.95***
HOP+STER	0.29

Notes: 1) PAHs only include common species between locations

Ascorbate and DHBA

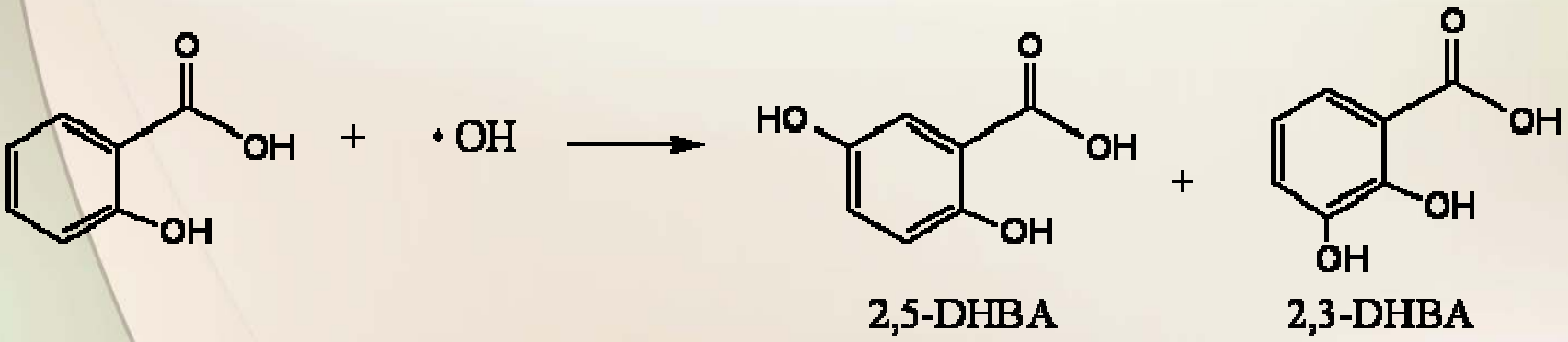
$$Asc + 2\,PM \longrightarrow Asc_{ox} + 2\,PM^{\bullet -}$$

$$2\,O_2 + 2\,PM^{\bullet -} \longrightarrow 2\,PM + 2\,O_2^{\bullet -}$$

$$2\,O_2^{\bullet -} \longrightarrow O_2 + H_2O_2$$

Monitor Asc consumption

$$Fe^{II} + H_2O_2 \longrightarrow {}^{\bullet}OH + Fe^{III} + H_2O$$



2,5-DHBA

2,3-DHBA

Monitor DHBA formation

Ascorbate/dihydroxy benzoate

	Ascorbate Consumption**			DHBA formation		
Compound	nmoles/nmoles/min			nmoles/nmoles/min		
	w/oDTPA	**w/DTPA***	**inhibition**	**w/oDTPA**	**w/DTPA**	**inhibition**
1,4 Naphthoquinone	**1.984**	**1.9607**	**1.20%**	**0**	**0**	**NA**
Menadione	**0.1266**	**0.1183**	**6.60%**	**0**	**0**	**NA**
9,10 Phenanthroquinone	**3.0524**	**2.9174**	**4.40%**	**0**	**0**	**NA**
Ferrous Ammonium sulfate	**0.5523**	**0.0063**	**98.90%**	**0.0051**	**0.0002**	**96.10%**
Cupric sulfate	**1.7201**	**0**	**100%**	**0.0205**	**0.001**	**95%**

**Conditions: AA(500µM),SA(50µM),pH 7.4; * Diethylenetriaminepetaacetic acid

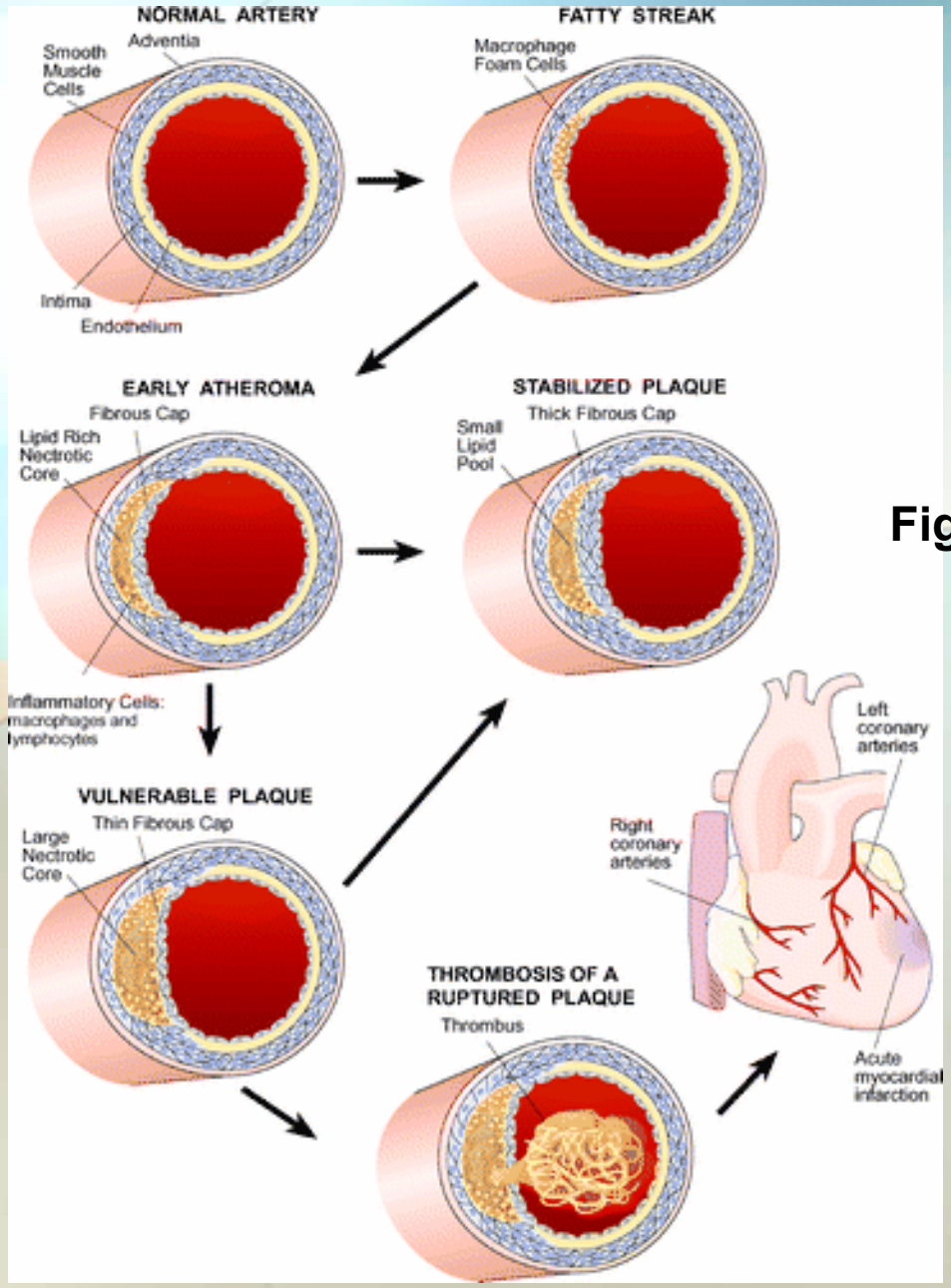


Figure 1 Stages of atherosclerosis.

Lusis, A. J., R. Mar, et al. (2004). "GENETICS OF ATHEROSCLEROSIS doi:10.1146/annurev.genom.5.061903.175930." Annual Review of Genomics and Human Genetics **5**(1): 189-218.

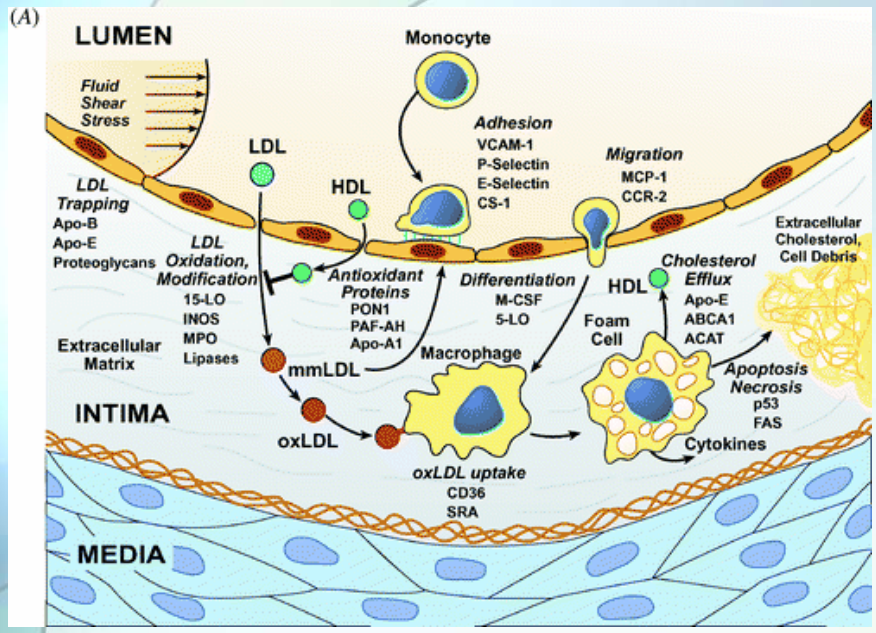
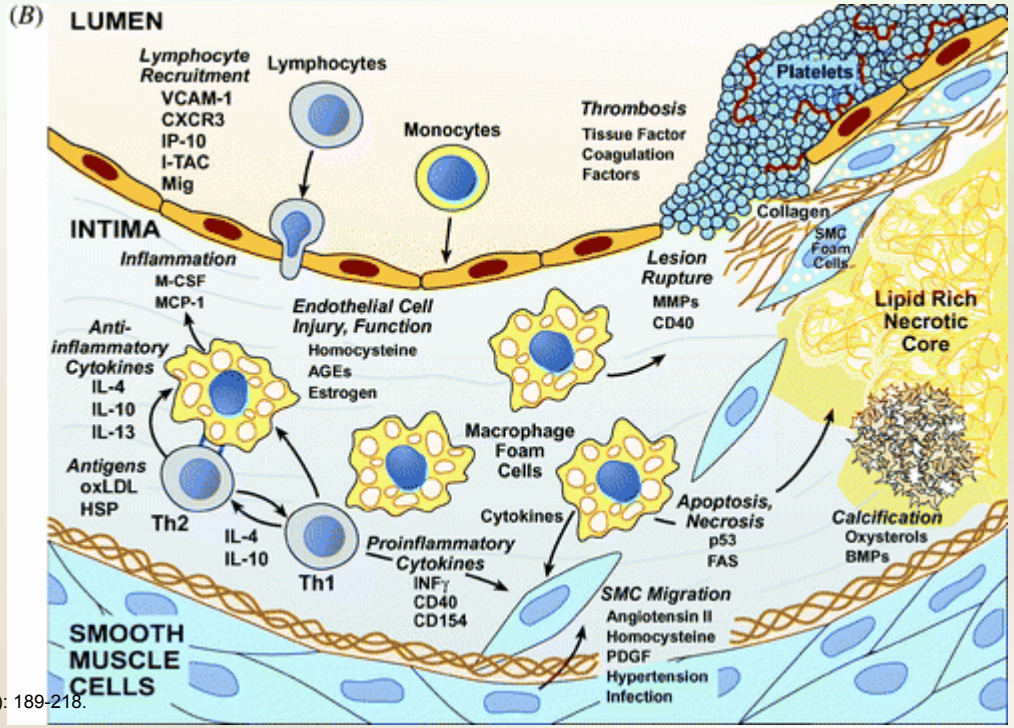


Figure 2 Molecular and cellular interactions in early (*A*) and late (*B*) stages of atherosclerosis.



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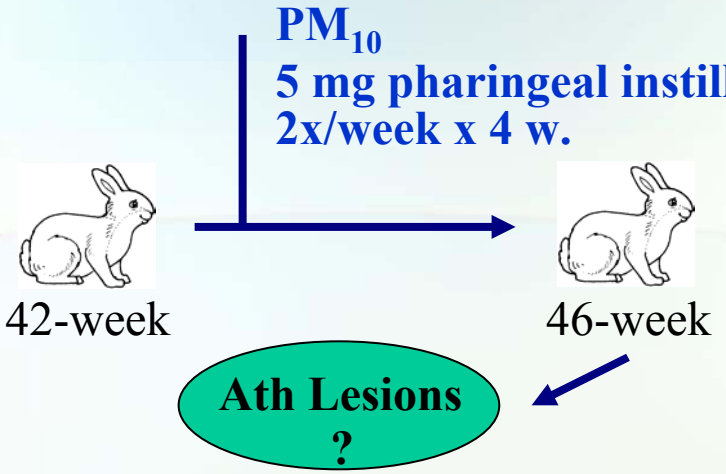
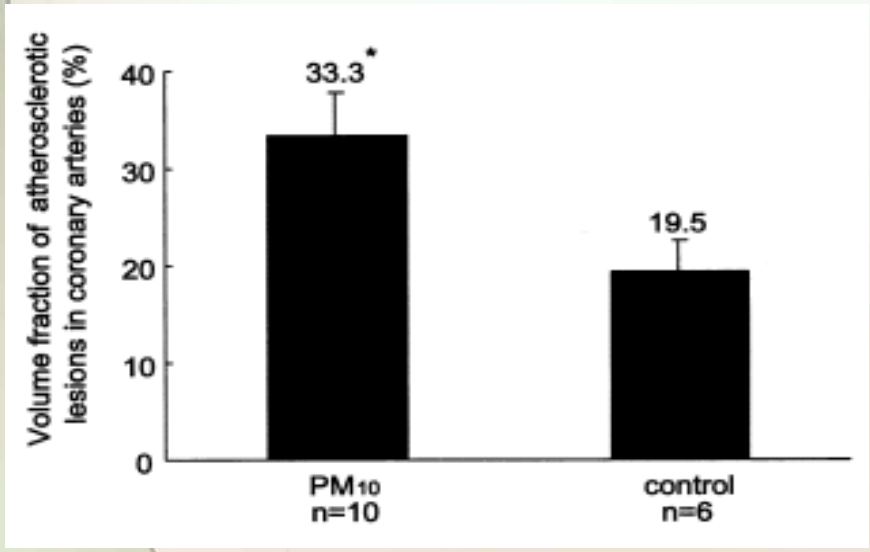
PM & atherosclerosis: animal models

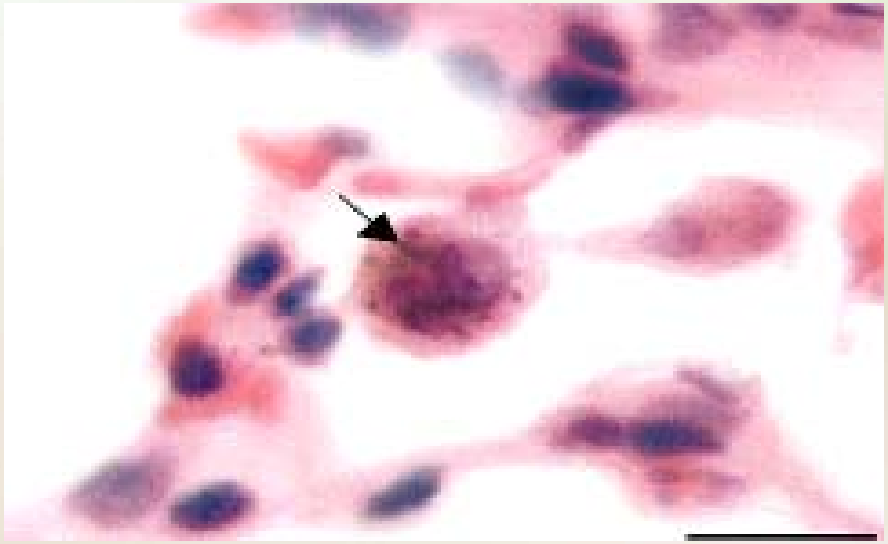
1) PM_{10} in Watanabe rabbits (***Suwa et al, 2002***)

2) $PM_{2.5}$ in $apoE^{-/-}$ mice (***Chen & Nadziejko, 2005)***
(Sun et al, 2005)

PM_{10} and atherosclerosis in rabbits







Suwa et al, JACC 2002; 39: 935

$PM_{2.5}$ and atherosclerosis in apoE null mice

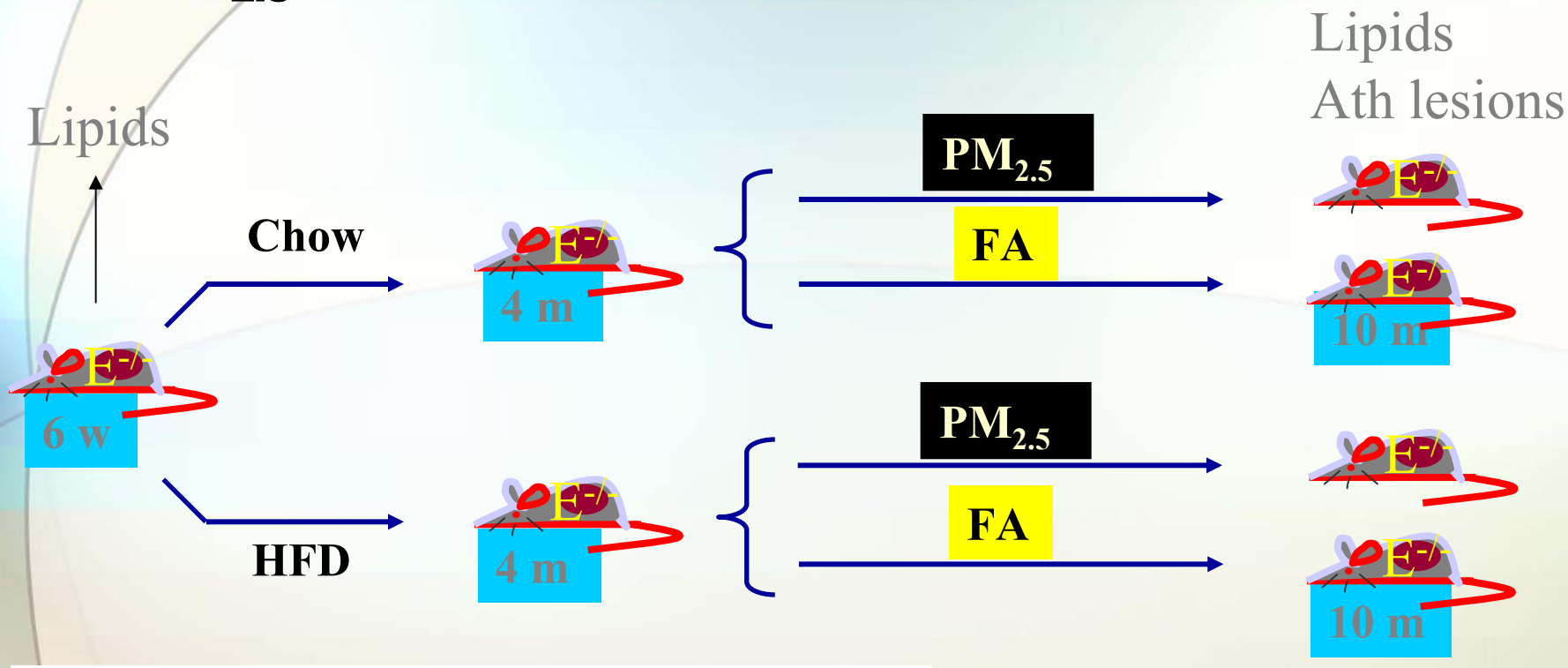


Table 3. Analysis of Plaque and Immunohistochemical Staining Parameters*

Staining	Normal Chow, Mean (SD)		P Value†
	Filtered Air	$PM_{2.5}$	
Plaque area, %	13.2 (8.1)	19.2 (13.1)	.15
Oil red-O	10.0 (4.1)	15.3 (11.8)	.13
CD68	7.0 (2.2)	12.8 (3.7)	<.001
3-Nitrotyrosine	1.1 (0.8)	4.4 (1.5)	<.001
Endothelial NOS	0.6 (0.3)	1.1 (0.5)	.06
Inducible NOS	0.8 (0.5)	3.2 (0.9)	<.001

Sun et al, JAMA 2005; 294: 3003

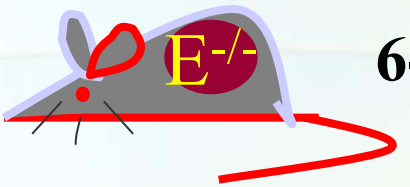
What is the effect of ultrafine particles on atherosclerosis?

Hypothesis:

PM synergizes with known proatherogenic stimuli and mediators in their ability to elicit oxidative stress and promote atherosclerosis. Most of the proatherogenic potential resides in the ultrafine particles fraction, highly enriched in redox cycling or electrophilic PM chemicals

Mobile Laboratory: AIRCARE 1

Experimental Design: apoE$^{-/-}$ on chow diet



6-week-old male

Chow

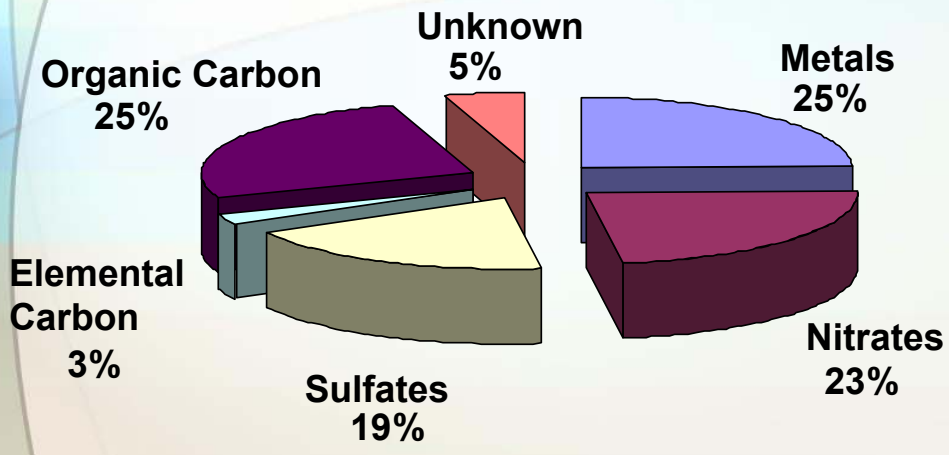
Exposures: 5 hours/session
3x/week for **5** weeks

Non-exposed (NE)	**Filtered air (FA)**	**$PM_{2.5}$ (FP)**	**$PM_{0.1}$ (UFP)**

* Aortic atherosclerosis assessment
* Lipid profile, plasma hydroperoxides, tissue gene expression

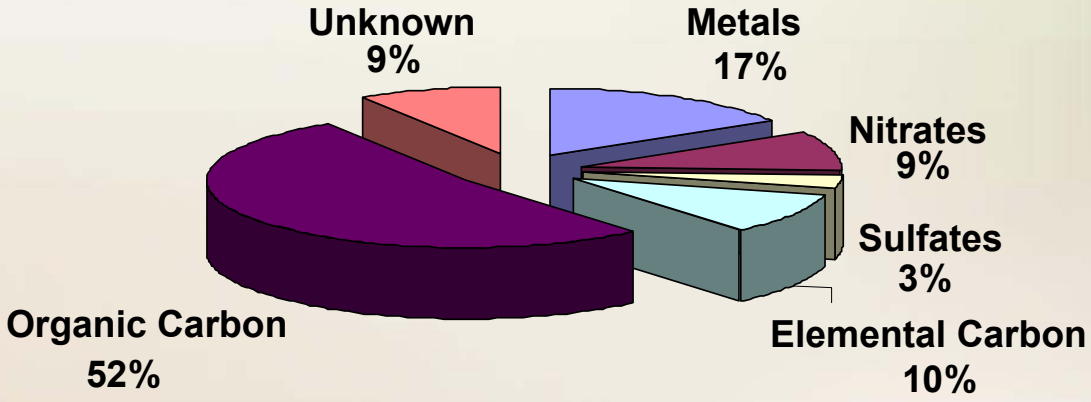
PM Composition

$PM_{2.5}$ Composition

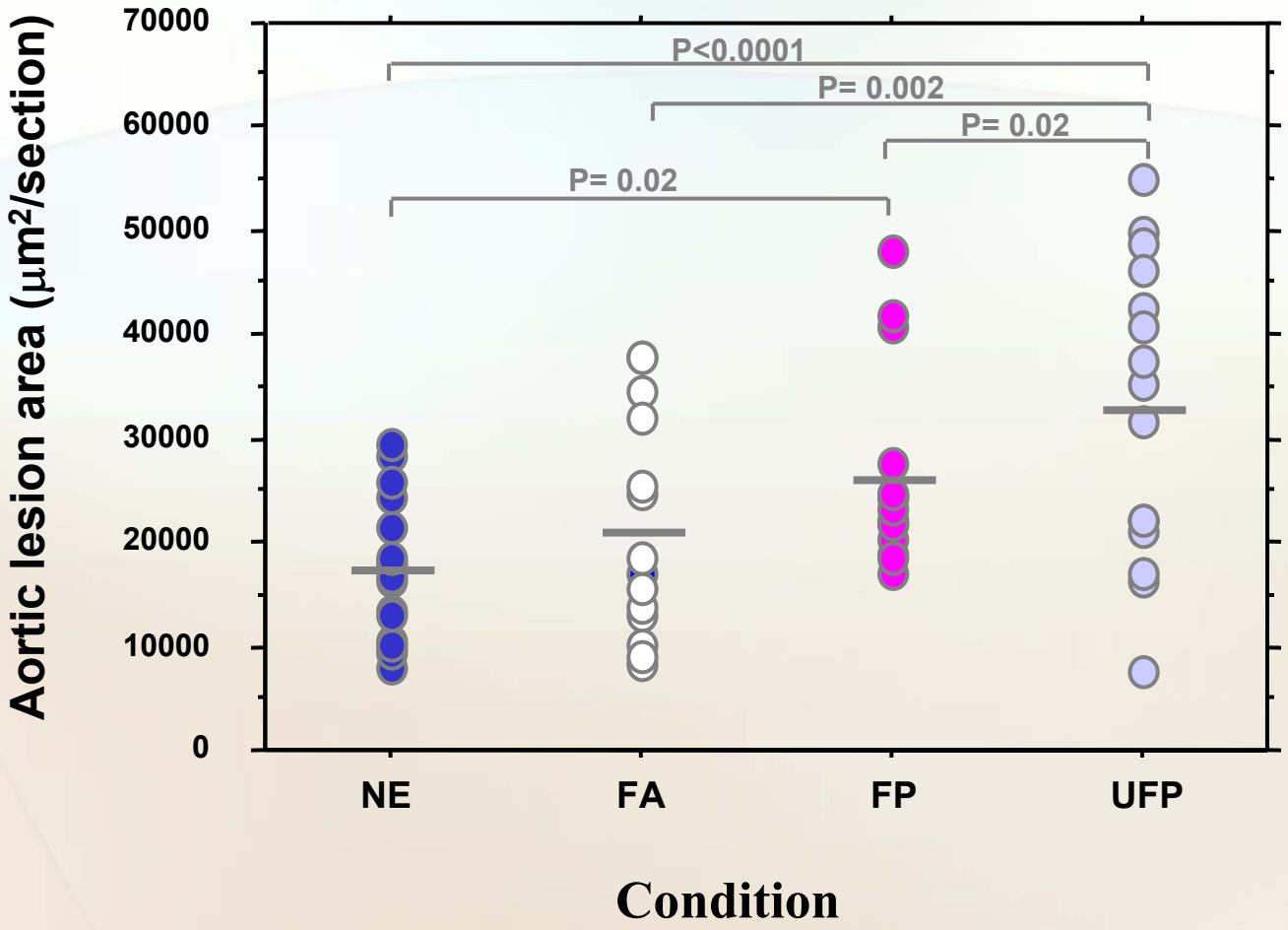


Simultaneous with this characterization-DTT assay for ROS, DHBA for hydroxyl radical and GAPDH for electrophilic chemistry, heme-oxygenase

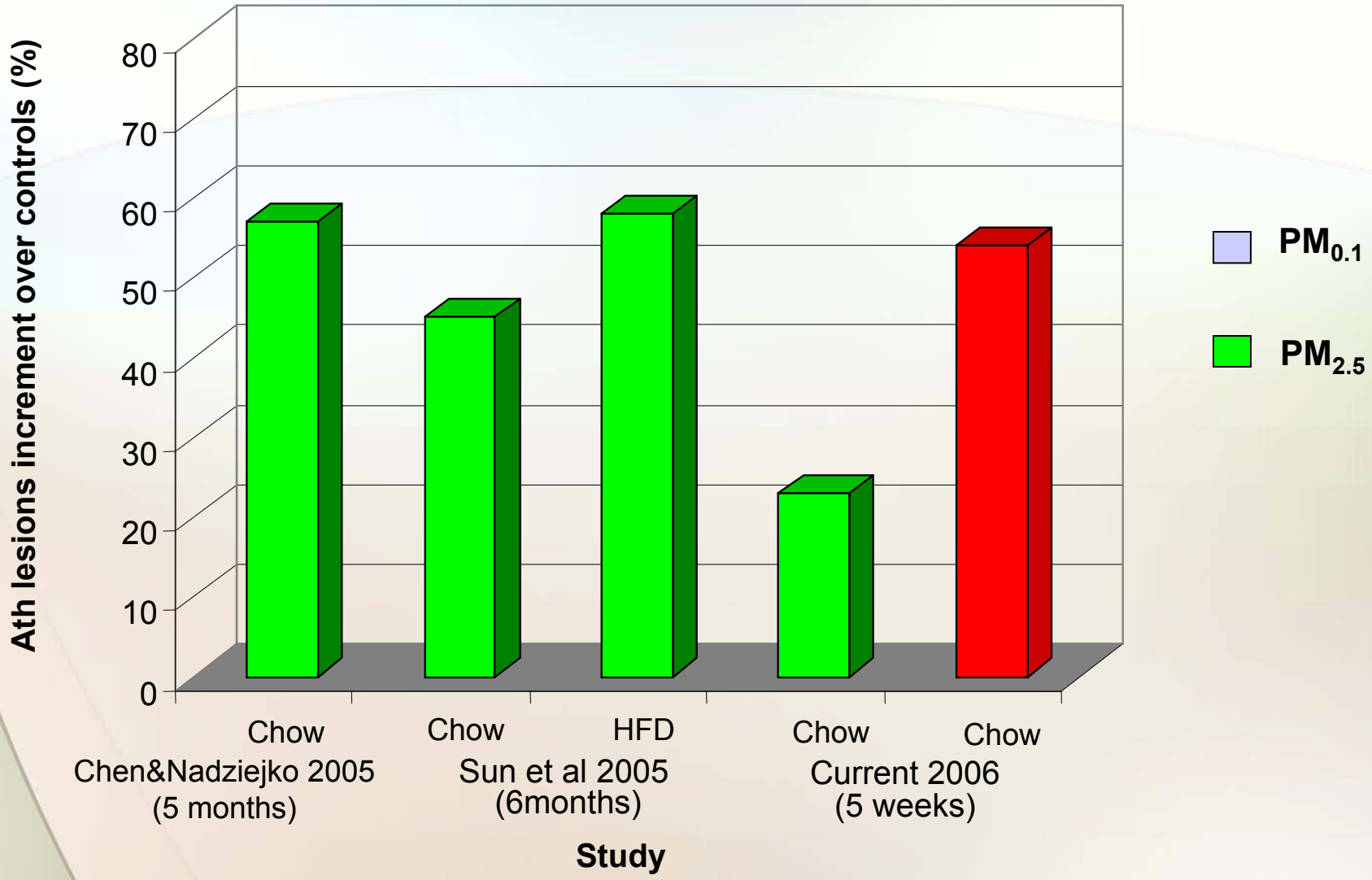
$PM_{0.1}$ Composition



Aortic atherosclerotic lesions



$PM_{0.1}$ is more proatherogenic than $PM_{2.5}$

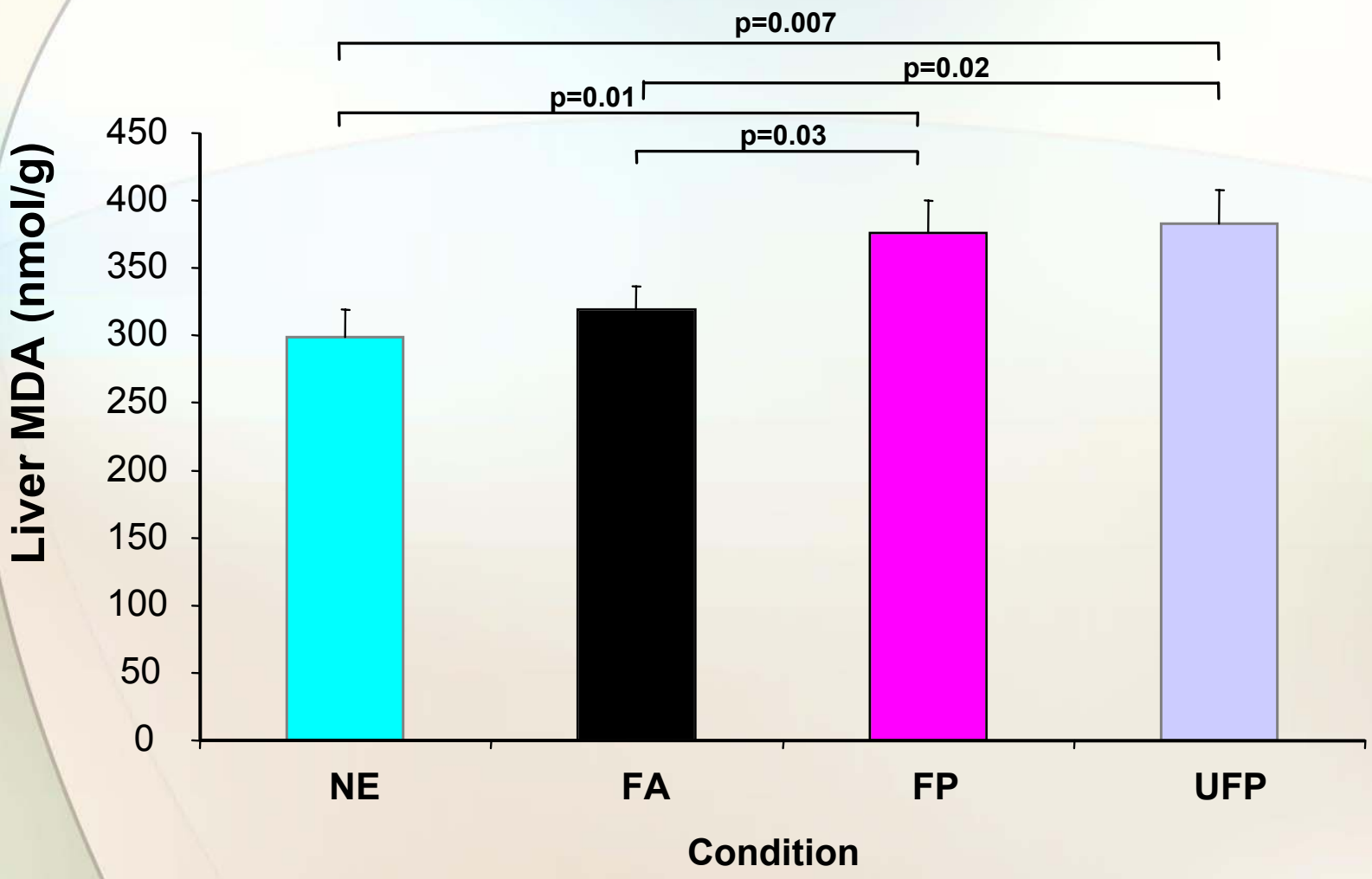


How does $PM_{0.1}$ promote atherogenesis?

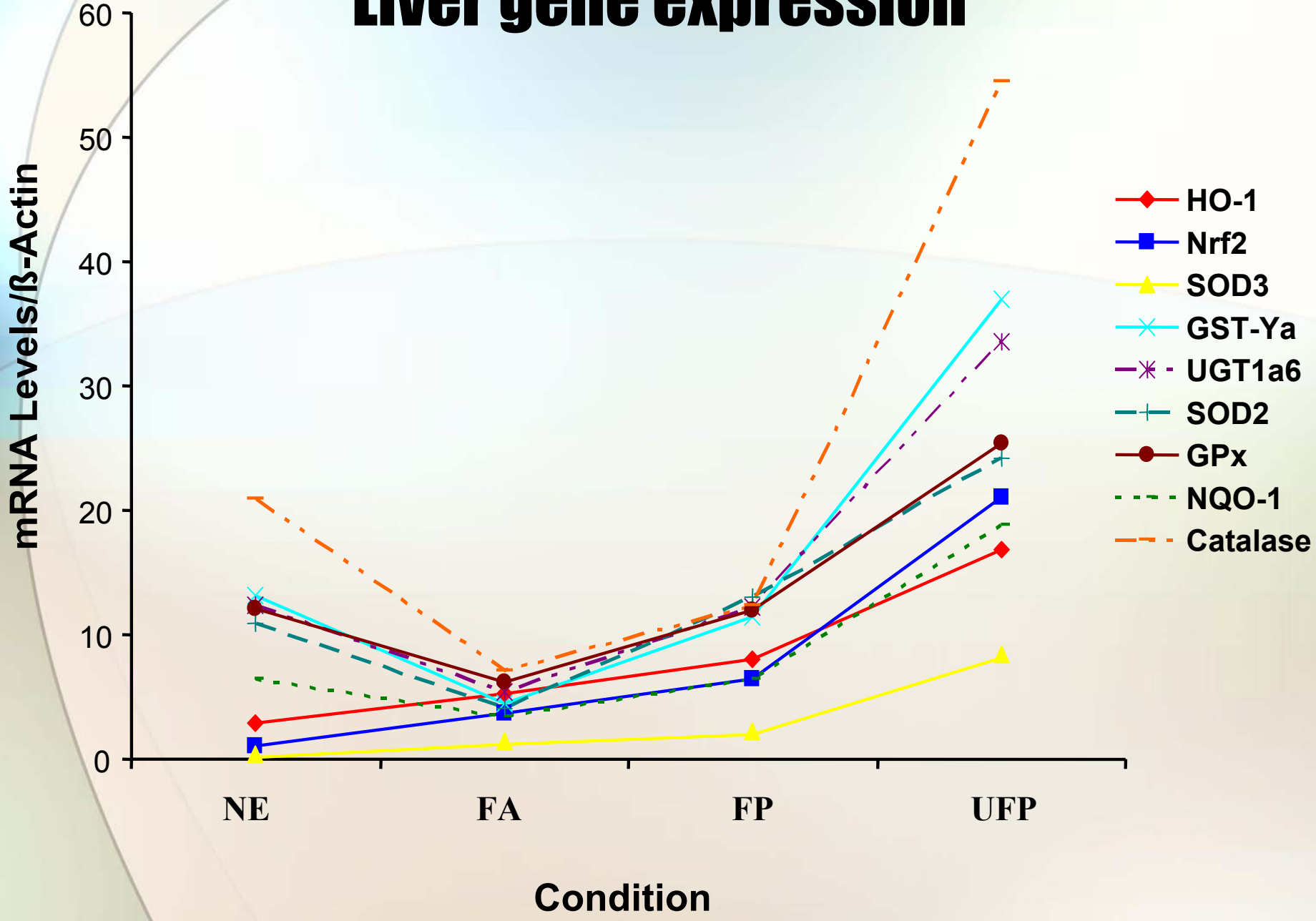
Plasma lipid levels

	Total cholesterol	HDL cholesterol
Baseline		
NE (n=18)	-	-
FA (n=17)	**349 +/- 13**	**11 +/- 1**
FP (n=17)	**355 +/- 13**	**11 +/- 1**
UFP (n=17)	**352 +/- 12**	**11 +/- 1**
End of protocol		
NE (n=18)	**372 +/- 15**	**8 +/- 1**
FA (n=17)	**397 +/- 13**	**9 +/- 1**
FP (n=17)	**459/- 21**	**9 +/- 1**
UFP (n=17)	**402 +/- 19**	**8 +/- 1**

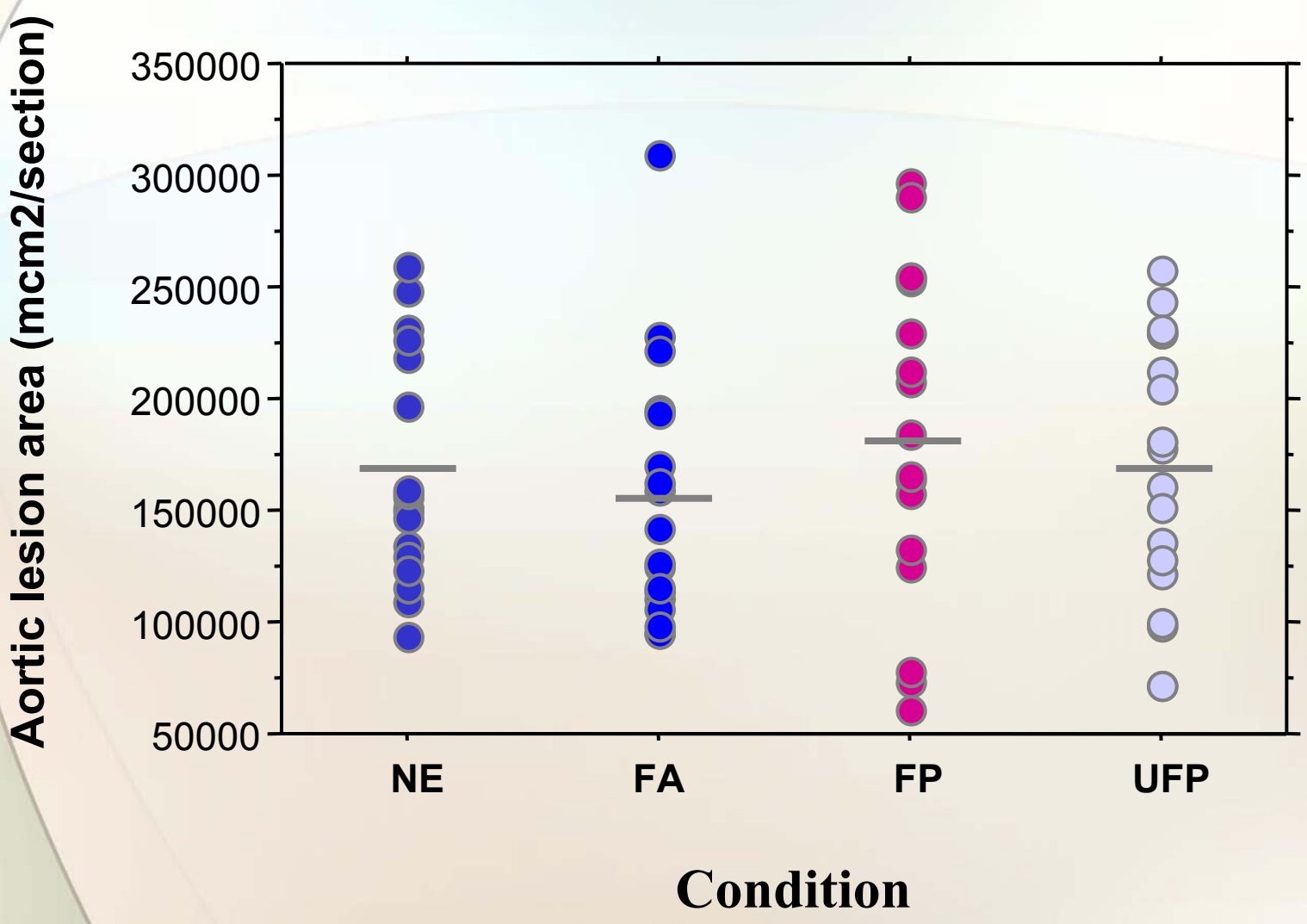
$PM_{0.1}$ induces systemic oxidative stress: Liver MDA



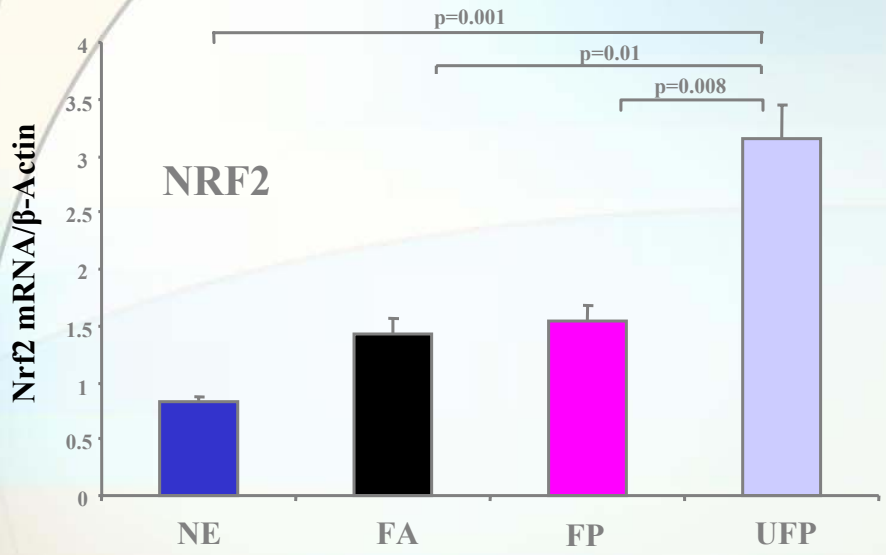
Liver gene expression

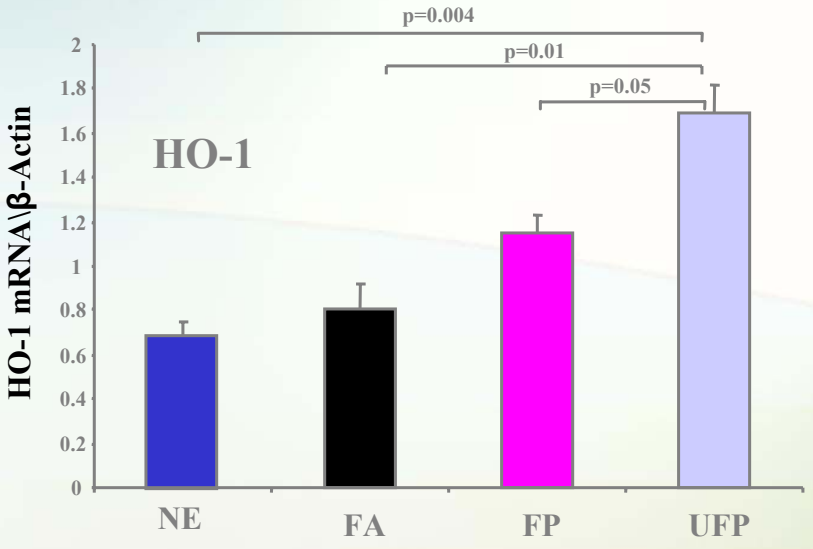


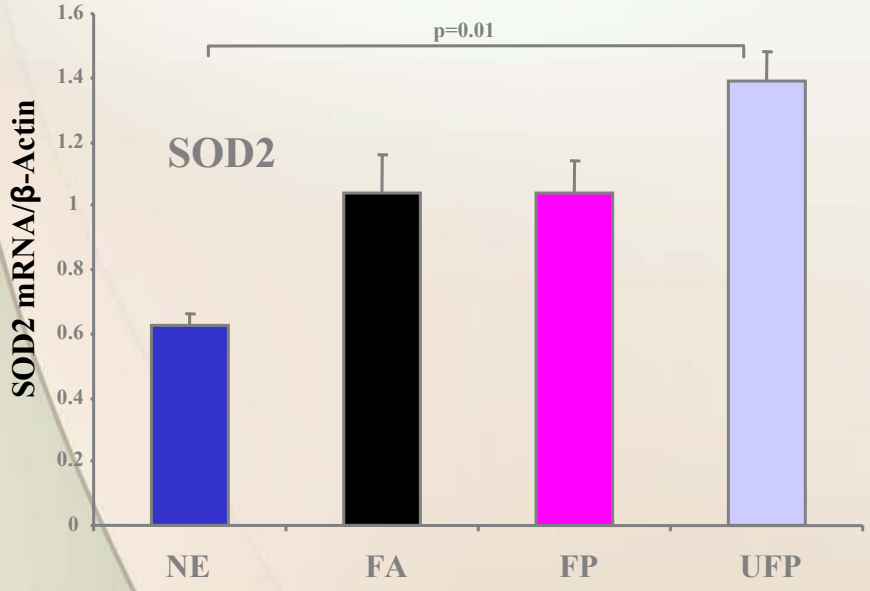
ApoE null mice on high fat diet: Aortic lesions

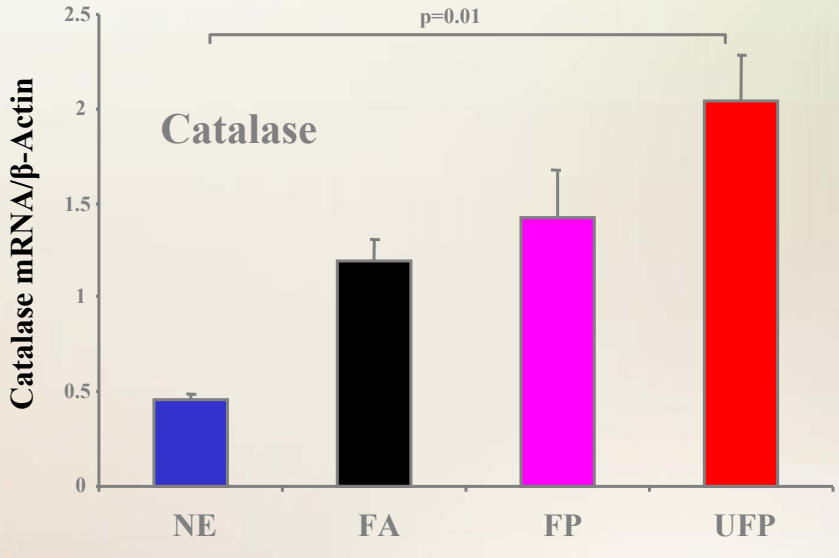


$PM_{0.1}$ also induces systemic antioxidant genes in HFD-fed mice: Liver gene expression









Summary

- **$PM_{0.1}$-exposed mice develop greater atherosclerotic lesions than all other mice (NE, FA, FP)**
- **Ultrafine particles lead to the lost of the protective antiinflammatory profile of plasma HDL without altering the lipoprotein level.**
- **$PM_{0.1}$ exposure results in increased systemic oxidative and systemic induction of Nrf2 and Nrf2-regulated antioxidant genes.**
- **The ultrafine particles fraction concentrates the air pollution-related proatherogenic effects.**
- **Ultrafine particles constituents synergize with known proatherogenic mediators in the induction of a large number of genes relevant in vascular inflammatory processes.**
- **Ulftrafine particles lead to a systemic effect characterized by increased oxidative stress and lost of the anti-inflammatory properties of HDL.**

Introduction and Outline

Cardiovascular Disease

- Hypothesis and Description
- Effects of Particles on the Heart

Effects on the Brain

- Description
- Inflammatory Responses

Geriatric rat exposures near the freeway

- Previous studies had shown that the ‘geriatric’ rat was sensitive to particle-induced inflammation and hemodynamic effects.
- The geriatric rat may be a useful model for PM effects in a sensitive human population.

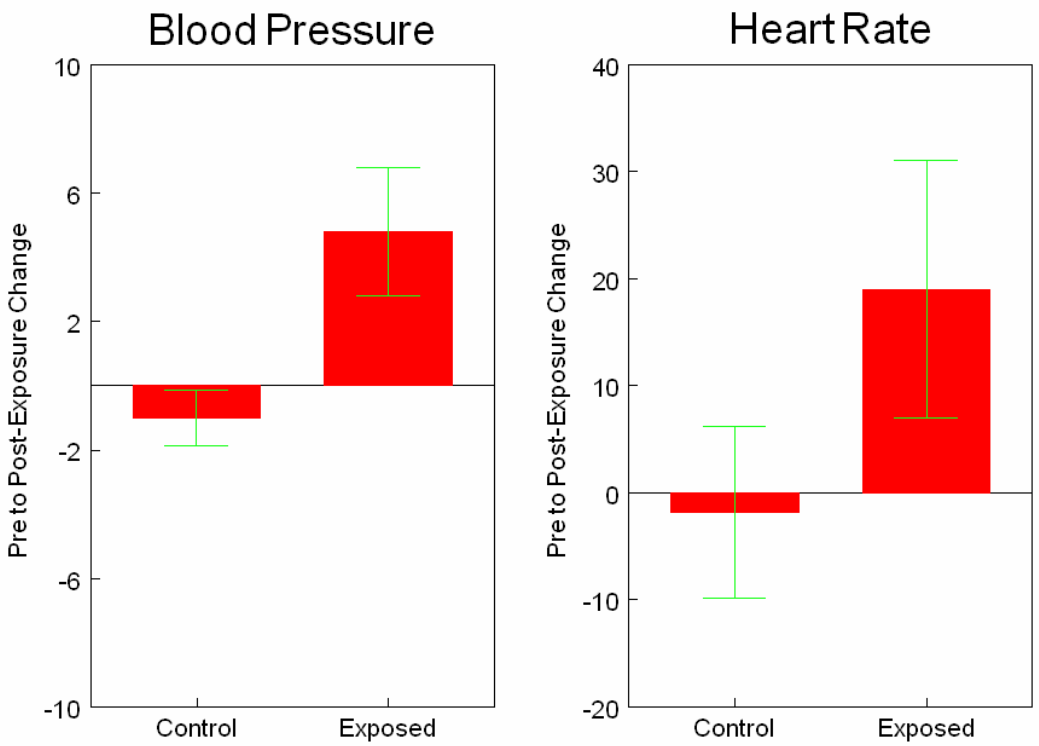
Approach

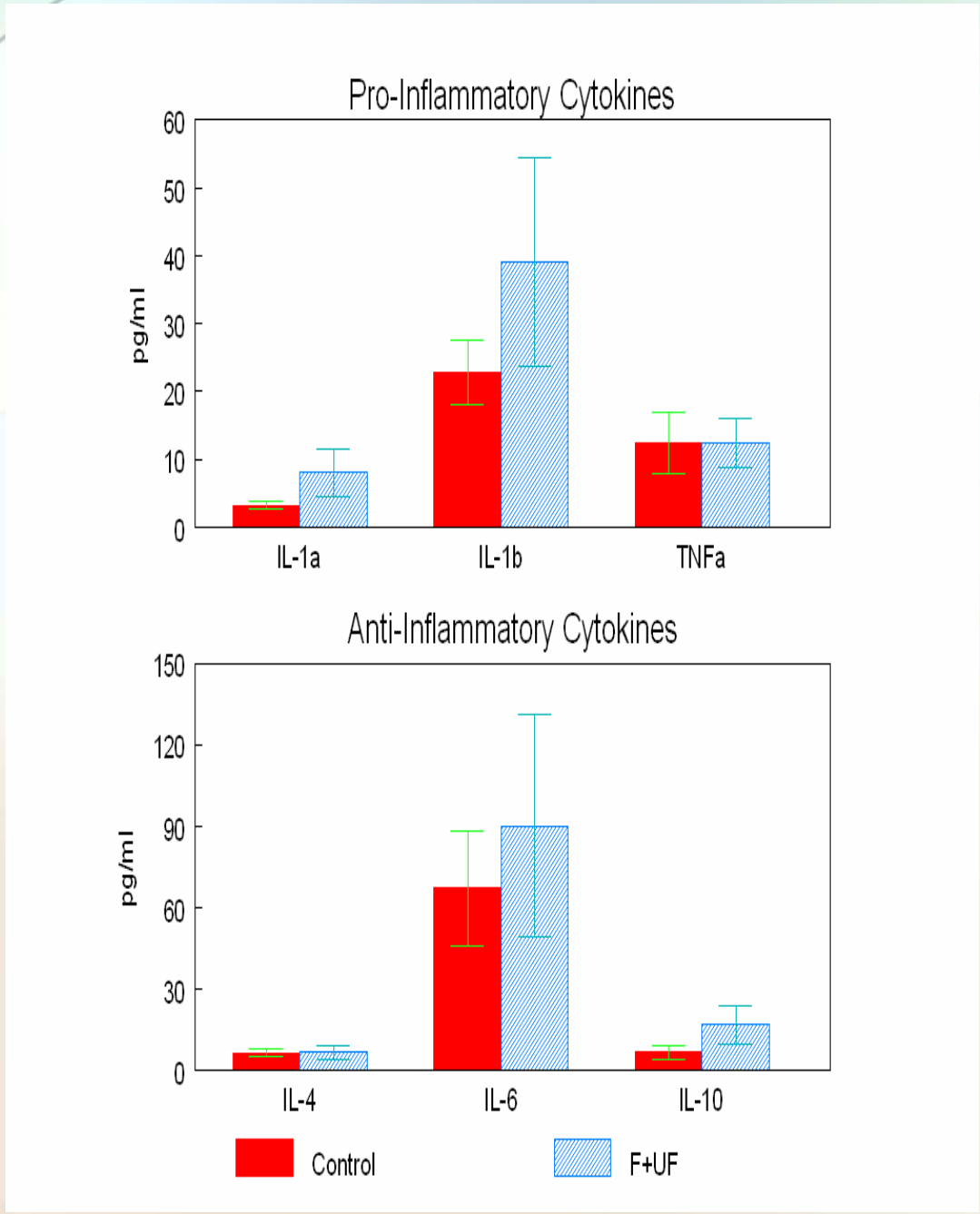
- Rats aged 24-26 months were exposed to the highest achievable F particle concentration.
- Exposures were 4 hours per day for 3 consecutive days. There were 8 controls and 8 exposed rats.
- 8 rats were implanted with blood pressure and EKG transponders. 4 were exposed to F CAPS and 4 to purified air.

Endpoints

- Animals were killed 24 hours after the last exposure.
- Lungs were lavaged. BAL was analyzed for cytokines.
- Macrophages were isolated and tested for free radical (superoxide) production.
- Telemetry Data were analyzed for HR, HRV and Arrhythmias.
- Blood pressure was measured using a tail cuff because of technical problems with the telemetry data.

Blood pressure and heart rate were increased after caps exposures

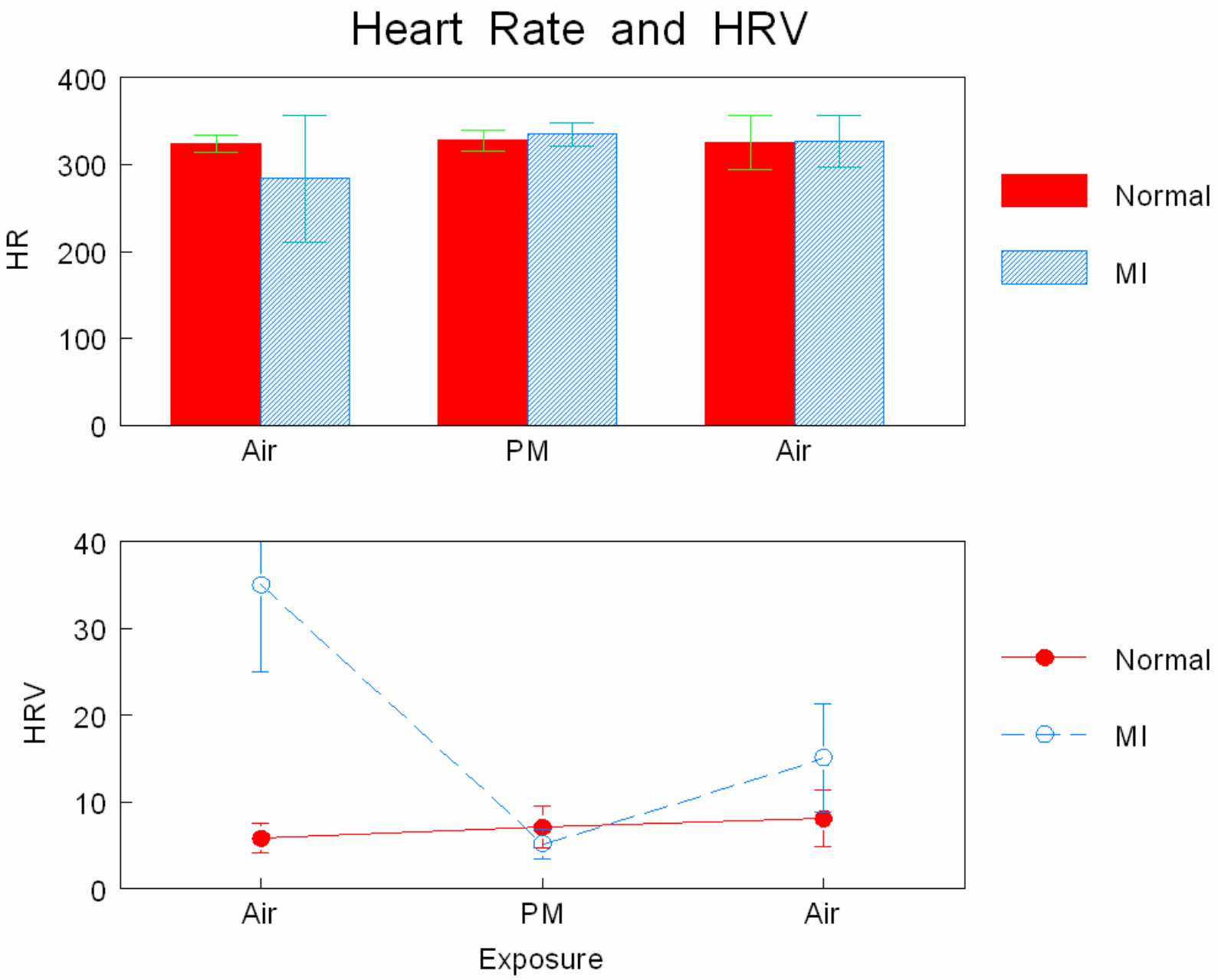



Pro-Inflammatory Cytokines
pg/ml
60
50
40
30
20
10
0
IL-1a
IL-1b
TNFa
Anti-Inflammatory Cytokines
pg/ml
150
120
90
60
30
0
IL-4
IL-6
IL-10
Control
F+UF

Cardiac function studies

In Vivo Studies

- Acute Exposures (5 hr per day, 3 consecutive days)
- Endpoints: HR, Arrhythmias, HRV
- Key Findings: HR showed no significant exposure-related effect; arrhythmias were increased in some but not all animals; HRV unchanged during exposure in normal rats but significantly decreased in rats which had surgically-induced myocardial infarctions (MI).


Heart Rate and HRV
HR
400
300
200
100
0
Air
PM
Air
Normal
MI
HRV
40
30
20
10
0
Air
PM
Air
Exposure
Normal
MI

Cardiological function – Ex Vivo

- Particles instilled into a heart cause significant changes in hemodynamics
- Soluble extracts from these particles have no effect.
- Particles if they reach the CV system can directly affect the heart.

Cardiac Function Studies – Ex Vivo

Hemodynamic values obtained at baseline and 30 minutes *in vitro* in paced control hearts and hearts perfused with ultrafine particles isolated from industrial diesel particulate matter (UFID) and its soluble fraction (SF-UFID).

Group	HR (beats/min)	LVSP (mmHg)	LVEDP (mmHg)	+dP/dt (mmHg/sec)	-dP/dt (mmHg/sec)	Coronary flow (ml/min)
Control Baseline	**284.6 ± 7.6**	**96.7 ± 12.3**	**10.1 ± 1.1**	**3114 ± 506**	**1404 ± 201**	**13.9 ± 1.0**
Control 30 min	**285.4 ± 7.7 #**	**81.8 ± 10.4 *, #**	**11.1 ± 12.4**	**2825 ± 623 #**	**1205 ± 200 #**	**10.1 ± 1.4 *, #**
UFID Baseline	**268 1 ± 10.9**	**85.7 ± 4.0**	**8.3 ± 3.7**	**2365 ± 158**	**1167 ± 273 †**	**13.7 ± 0.8**
UFID 30 min	**187.9 ± 124.6 †**	**37.9 ± 20.3 *, †**	**5.9 ± 6.2**	**1188 ± 858 *, †**	**522 ± 427 †**	**3.3 ± 2.9 *, †**
SF-UFID Baseline	**275 6 ± 7.4**	**100.0 ± 15.1**	**9.7 ± 1.3**	**3081 ± 286**	**1559 ± 216**	**12.6 ± 0.5**
SF-UFID 30 min	**275.8 ± 7.2**	**77.8 ± 35.2 ***	**5.7 ± 1.6**	**2853 ± 1221**	**1170 ± 637**	**8.4 ± 2.8 ***

Results are shown as mean ± SD.

* - $p<0.05$ vs. baseline within corresponding group; # - $p<0.05$ vs. UFID-treated group at the same timepoint;

† - $p<0.05$ vs. soluble fraction-treated group at the same timepoint.

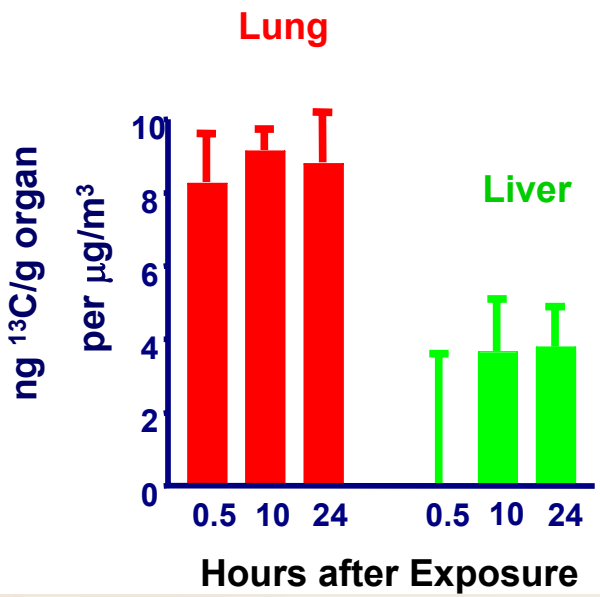
n = 8 in the control group, 6 in the UFID-treated group, and 5 in the SF-UFID-treated group

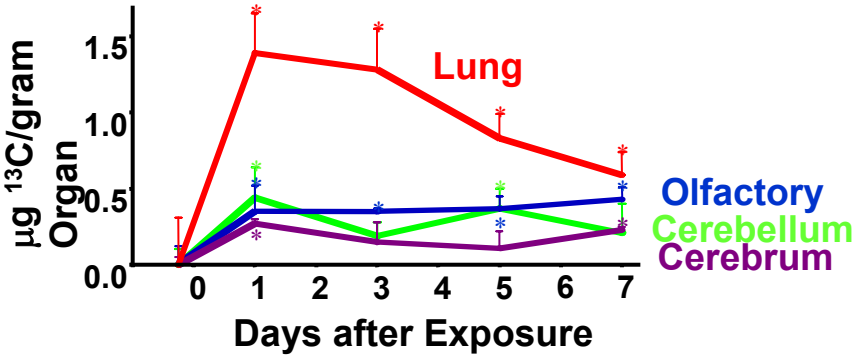
Inflammatory mediators in the brain

- There have been speculations that non-familial neurodegenerative diseases are:
  - related to exposures to environmental toxins
  - at least, in part, due to oxidant-related mechanisms
- Dogs in exposed to air pollution in Mexico City exhibit lesions in brain tissue.
- Brain tissues from mice exposed at BH2 were analyzed for expression of IL-1, TNF and NFkB.

Does PM affect other organ systems?

Labeled ultrafine particles are translocated to the lung and to specific regions of the brain, where they stimulate brains cells to produce pro-inflammatory markers





Brain Inflammation Markers Tissue from Mice Exposed at BH2 2002			
	Control	UF	F+UF
TNFα (ng/mL)	2.0±0.1	2.2±0.1	2.5±0.2
IL-1α (ng/mL)	1.6±0.2	2.7±0.3*	2.0±0.4*
NF_kB (units x 10^{-3})	8.5±4.4	11.0±1.6**	10.7±3.0**

Sources: Campbell et al, Neurotoxicology, 2005; Oberdorster et al., EHP, 2005

Summary

- ***Geriatric rats exposed near the roadway showed:***
  - **Changes in HR, BP and macrophage responses were still seen.**
  - **HRV was significantly changed in a second study**
  - **Hemodynamic parameters changed**
  - **Evidence for inflammation and oxidative stress**

- ***Significant inflammatory responses were observed in brain tissue 2 weeks after CAPs exposures near the roadway but not at a remote site.***
  - **Possibly of relevance to the role of environmental agents in promoting neurological diseases such as Parkinson's.**
  - **Responses at a site remote from freeways were not significantly different from controls.**

Key questions for future work

- **Which sources pose the greatest risks to public health?**
  - Need for studies of the relationships among specific sources, including mobile sources, atmospheric chemistry products, wood smoke, cooking and others, and toxicity-health effects

What are critical characteristics of PM in relation to toxicity?

- Further evaluation of size fractions needed
- Relationship between toxic mechanisms and specific toxic components

Which health effects are most sensitive to low levels of PM?

- More quantitative exposure-response data are needed
- Role of susceptibility findings including gene-environment interactions in determining most sensitive endpoints
- Delfino's human panel studies will provide the human linkage between cardiovascular disease and the other four elements of our research, namely characterization, chemical assays, biological roadmap, and human studies as well as Gong's clinical studies and CHS